ACUTE METABOLIC EFFECTS OF PHYSIOLOGIC ELEVATIONS OF PLASMA GROWTH HORMONE

Gerri Anne Schulman

1980

ACUTE METABOLIC EFFECTS OF PHYSIOLOGIC ELEVATIONS OF PLASMA GROWTH HORMONE

Gerri Schulman

A THESIS SUBMITTED TO THE YALE UNIVERSITY SCHOOL OF MEDICINE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF MEDICINE

1980

ACKNOWLEDGEMENTS

I would like to thank the following people for their guidance, support and assistance: Robert Sherwin, M.D., William Tamborlane, M.D., Rosa Hendler, M.D., Mary Walesky, R.N., Ralph Jacobs and Thomas Meehan.

TABLE OF CONTENTS

ABSTRACT

Pharmacologic doses of growth hormone (HGH) or chronic HGH excess induce glucose intolerance and insulin resistance. Whether acute increments of HGH are diabetogenic as well, is not established. In fact, HGH is reported to have acute insulin-like effects. In this study, 8 postabsorptive normal subjects randomly received a HGH (3μg/kg/hr) or saline infusion for 8 hours. After 5 hours, a 100 gram oral glucose tolerance test (OGTT) was performed and HGH or saline was continued for the remaining 3 hours. Insulin binding to erythrocytes and monocytes was measured before and 4 hours after HGH infusion. Plasma HGH during HGH infusion rose to 25-35 ng/ml. Before OGTT, plasma glucose remained unchanged (85±2 mg/dl vs 86±2 at 5 hours), FFA increased by 50% ($p<0.01$) and ketones increased by 86% ($p<0.01$), despite 35% greater fasting plasma insulin levels in the HGH-infused subjects ($p<0.005$). Fasting plasma alanine decreased by 22% ($p<0.005$) during HGH infusion whereas the concentrations of other amino acids remained unchanged. After OGTT, HGH-infused subjects exhibited a marked deterioration in glucose tolerance. Plasma glucose rose by 30-50 mg/dl ($p<0.01$) above saline control values (2 hours, 162±12 mg/dl vs 121±7 and 3 hours, 124±13 vs 85±6) and the area under the glucose response curve increased 2-fold ($p<0.005$). This occurred in the face of 2-fold higher insulin levels (peak values 168±33 μU/ml vs 85±26, $p<0.005$) in the HGH-infused group. Insulin binding to erythrocytes decreased by 16% ($p<0.01$), whereas, insulin binding to monocytes increased by 45%. Conclusions: 1) Acute physiologic elevations in HGH, which fail to alter fasting plasma glucose, cause glucose intolerance and hyper-

insulinemia in man: 2) HGH-induced insulin resistance occurs in spite of a rise in insulin binding to monocytes. Our data suggest that HGH has rapid-onset insulin antagonistic effects which might contribute to glucose intolerance during stress.

INTRODUCTION

The metabolic consequences of stress in man frequently include deterioration of glucose tolerance, protein wasting, enhanced lipolysis and ketosis (1). In addition, stress can provoke elevations of one or more hormones, including epinephrine, glucagon, cortisol, and growth hormone (2). The roles of the first three of these hormones in mediating stress hyperglycemia have recently been examined (3). However, the significance of acute physiologic elevations of growth hormone is not known.

The purpose of this thesis is to examine the effects of acute physiologic increments in growth hormone levels on carbohydrate, fat, and protein metabolism. As background information, the results of previous studies in both man and animals will be reviewed. First, the regulation of growth hormone secretion and the growth hormone response to stress will be discussed in order to establish the magnitude and duration of the growth hormone response and the type of situations in which this response might be expected to occur. Then the effect of chronic elevations of growth hormone, as seen in acromegaly, will be considered. Finally, the effects of more acute growth hormone elevations on fat, glucose and protein metabolism will be summarized. When considering this data, it will be important to remember that most of it derives from studies in which supra-physiologic concentrations of growth hormone were achieved.

GH SECRETION

Growth hormone (GH) is a polypeptide secreted by the pituitary in response to a variety of physiologic and psychologic stimuli. Basal plasma levels in adults normally range between 1 to 5 ng/ml (4,5). Bursts of GH secretion occur throughout the day. The highest and most consistent increases in GH are seen during stage III-IV sleep with peak levels averaging 34 ± 6 ng/ml (6).

Basal GH concentrations have been reported to be slightly higher in pre-menopausal women than in men or post-menopausal women (7). When the magnitude of the GH response to exercise, arginine or insulin-induced hypoglycemia is compared at different stages of the menstrual cycle, significantly higher peak GH levels are found in the high estrogen phases of the cycle than during menstruation (8-10). Administration of estrogen but not progesterone or testosterone to men also augments the peak GH response to various stimuli (8,9). Estrogens do not effect the metabolic clearance rate, distribution space, or plasma half life of growth hormone (11). They seem to work by increasing the sensitivity of the pituitary to growth hormone releasing factor (12).

Regulation of GH secretion is closely tied to fluctuations in plasma glucose concentrations. Hypoglycemia is a potent stimulus of GH secretion. It is well documented that insulin-induced hypoglycemia causes increments of 30 to 50 ng/ml in plasma GH that persist for several hours (5,13-16). Insulin by itself does not mediate this response since administration of

glucose with insulin prevents GH increases (13). Administration of 2-deoxy-D-glucose, an inhibitor of intracellular glucose utilization, results in hyperglycemia but increased GH levels suggesting that intracellular glucose concentration determines the GH response (14). Conversely, glucose administration suppresses plasma GH concentrations in normal subjects (5,13). Results of animal studies localize this response to glucose receptors in the ventromedial nucleus of the hypothalamus (10). These receptors respond to absolute hypoglycemia, changing blood glucose concentrations and intracellular deprivation of glucose (10).

Exercise induced augmentation of GH secretion has been reported by several groups (5,15,17). GH has been shown to increase during the first hour of walking on a treadmill to 43 ng/ml and then to slowly decrease after that despite continuing exercise (17). Although blood glucose levels did not change significantly during this study, administration of glucose did suppress the exercise-induced rise in GH (17).

GH secretion is augmented in response to a variety of physical stresses. Several studies have documented a rise in GH during surgery (15,18-21), the magnitude and duration of which is proportional to the degree of surgical stress (22). Patients undergoing minor procedures (inguinal herniorrhaphy) generally fail to exhibit GH elevations, whereas patients undergoing major surgery (aortofemoral bypass) demonstrate peak intraoperative GH levels of 20 to 30 ng/ml with elevated levels in blood lasting up to eight days post-operation (22). Other invasive procedures that augment GH secretion include arterial catheterization (23)

and cardiac catheterization (24).

Acute infectious diseases are associated with elevated GH levels. Injection of pseudomonas-derived polysaccharide leads to peak GH levels of 26 ± 2 ng/ml at 2 hours (25-27). This is not secondary to fever since injection of etiocholanolone, a pyrogen, does not augment GH secretion (27). Additionally, the increase in GH during injection of pseudomonas polysaccharide is not suppresible by glucose (25). Hypersecretion of GH is also seen in viral illnesses. Inoculation of normal subjects with serum containing the sandfly fever virus results in peak GH levels of 5 to 12 ng/ml (28). The rise in GH precedes the onset of clinical symptoms by 7 to 14 hours and persists for several days (28).

Diabetic ketoacidosis (DKA) is another acute condition in which elevated GH levels are frequently seen (7,29-31). Unger (7) found that in 5 patients with DKA, GH levels ranged from 1.0 to 70.0 ng/ml with the highest values seen in the most ketotic patients. Cryer and Daughaday (29) described elevated GH levels, ranging from 13 to 215 ng/ml, in 7 of 12 patients with DKA. They saw no correlation between the magnitude of the GH response and the clinical severity, degree of hyperglycemia or depression of serum bicarbonate. However, they did not directly measure serum ketones and try to correlate that with GH response. Not all studies have shown elevated pretreatment GH levels (32-33). Instead, GH levels were seen to rise after initiation of treatment to levels ranging from 30 to 250 ng/ml and to remain elevated

for at least 5 or 6 hours (29,32,33).

Elevation in GH levels are associated with more chronic disease in man. Patients with Laennec's cirrhosis have been shown to have abnormally high GH levels (34-36). Hernandez et al.(35) found increased fasting GH levels, ranging from 6.5 to 28 ng/ml, in 4 of 8 patients with cirrhosis. Conn and Daughaday (36) also reported elevated GH levels, ranging from 4 to 33 ng/ml, in 14 of 18 patients. Those patients with normal GH valueshad clinically and chemically less advanced disease. Similar results are seen in chronic renal failure (37-39). For example, Wright et al.(39) found GH elevated up to 60 ng/ml in 50 percent of their patients. In both chronic liver and renal disease, the abnormally high GH levels are partially due to a decreased metabolic clearance rate and increased half life of the hormone (11). Additionally in liver disease hyperestrogenemia might potentiate secretion (12,35). There is also evidence that hypothalamic regulation is altered. Paradoxical responses frequently occur after standard provocative testing in patients with chronic liver and kidney disease. It has been reported that GH increases after glucose administration (36-39) and fails to rise after tolbutamide-induced hypoglycemia (40) in such patients.

Psychological stress is also associated with elevations in GH. Neurotic subjects showed significant increases in GH during the mirror drawing test, a standard procedure for inducing psychological stress (41). In another study, a medical student

told that he had received an injection of a large amount of insulin had an increase in GH by 1 hour to 10.5 ng/ml while four students given the same saline injection but not told that it contained insulin had no rise in GH (42). Subjects told that they were going to see a stressful movie also showed significant increases in plasma GH (43). Several studies support the idea that elevations in GH in response to stress are related more to the individuals type of defense mechanism than to the particular type of stress and are independent of change in the adrenocortical response (24,43-44).

The secretion of GH is ultimately regulated by stimuli from the central nervous system. There have been several recent reviews detailing these interactions (4,10,45-46). In brief, pituitary synthesis and release of GH is under the control of the hypothalamus. Somatostatin has been shown to be the inhibitory hypothalamic factor (47). Although no hypothalamic stimulatory substance has yet been isolated, there is convincing evidence that one exists (12). In humans, the hypothalamus is thought to exert a predominantly stimulatory effect since hypothalamic lesions or section of the pituitary stalk result in lower basal GH levels and decreased GH response to hypoglycemia, arginine, and L-DOPA (4,45-46). Augmentation of GH secretion in response to insulin-hypoglycemia, arginine, vasopressin, exercise and surgery are blocked by phentolamine (20,45). Sleep related GH release is not effected by α or β adrenergic blockade. The site of action of neurotransmitter is thought to be the hypothalamus since

neuroantagonists have no effect on GH secretion induced by direct electrical stimulation of the hypothalamus but do block GH secretion after amygdaloid and hippocampal stimulation (45).

The release of these hypothalamic factors is mediated via input from other areas of the brain. In the rat, electrical stimulation of the ventromedial nucleus, preoptic area and corticomedial amygdala inhibit GH secretion, whereas stimulation of the hippocampus, basolateral amygdala, interpeduncular nucleus and locus coerulus augment GH secretion (48-49). Different areas of the brain might be involved in control of GH at rest than during stress since septal lesions do not effect basal or physiologic GH fluctuations but do potentiate the GH response to stress (50).

ACROMEGALY

The effects of chronic elevations of GH on carbohydrate and lipid metabolism are exemplified by the derangements seen in acromegaly. Clinically, the most obvious finding is the increased incidence of diabetes mellitus, which occurs in 20 to 25 percent of patients (51-52). The diabetes occurring in patients with acromegaly is characterized by relative insulin resistance and elevated fasting insulin levels (53-54). Additionally, the initial burst of insulin secretion seen after a glucose load in normal subjects and in non-diabetic acromegalics, is decreased in diabetic acromegalics although total glucose-stimulated insulin secretion may be normal (54). It has been suggested that the development of diabetes in acromegaly is a consequence of the failure of insulin secretion to keep pace

with the demands of insulin resistance (53,55). This hypothesis is supported by studies following acromegalic patients over time. In these studies, it was shown that individual patients with normal glucose tolerance demonstrated marked hyperinsulinemia, whereas diabetes developed when they became unable to increase their insulin output in response to glucose (55).

Even in those acromegalic patients without diabetes (defined as fasting hyperglycemia and/or glycosuria), there is evidence of decreased glucose tolerance. In several studies, intravenous glucose tolerance tests were performed on non-insulin dependent patients with normal fasting blood sugars. Their rate constant for disappearance of glucose from the blood (K) was compared with that of normal subjects. Ikkos el al. (56) found that 9 of 17 patients (53%) had a $K<1$, whereas all eleven controls had a $K>1$. Cerasi and Luft (57) found that 2 of 9 patients (22%) had a $K<1$ and that the average K in acromegalics and controls were 1.3 and 1.75, respectively. Sonksen et al. (55) similarly found a $K<1$ in 5 of 15 patients (33%) with an average K of 1.4 in the acromegalics and 2.6 in controls. Results of oral glucose tolerance tests are in agreement with the results of intravenous testing. Beck et al. (58), using the criteria of Fajans and Conn (glucose greater than 120 mg/dl at 2 hours), found abnormal glucose tolerance in 8 of 12 acromegalics (67%), while Fineberg et al. (59) found an average 2 hour glucose of 153 mg/dl in the 9 acromegalics studied and 102 mg/dl in controls. Since none of these studies compared patients with age-matched

controls, they may be overestimating the frequency of abnormal glucose tolerance. However, it is still evident that a significant proportion of acromegalic patients with fasting euglycemia are less efficient in disposing of a glucose load than normal subjects.

Although decreased glucose tolerance is frequently present in acromegaly, insulin levels are often elevated. Among patients with normal fasting blood sugar, 40 to 80 per cent have elevated fasting plasma insulin levels (55,57,59,61). Glucose-stimulated insulin secretion is increased approximately three-fold in 60 to 70% of patients including both those with normal and abnormal glucose tolerance (53,55,58,59,62). Insulin secretion in response to non-hyperglycemic stimuli such as arginine infusion or a protein meal is also elevated in acromegaly (59).

The presence of hyperinsulinemia without hypoglycemia or increased glucose tolerance suggests that chronic overproduction of GH might lead to a state of insulin resistance. This hypothesis is supported by studies in which insulin was infused into the brachial artery of patients with acromegaly and the arterio-venous difference in glucose concentration monitored. In these studies, insulin-induced increases in glucose uptake by muscle and adipose tissue were decreased between 33 and 50 per cent in acromegalic patients as compared to controls (63-65).

Changes in the binding of insulin to its receptor on monocytes are seen in acromegaly (66). Monocytes taken from patients with acromegaly have a decreased concentration of insulin receptor per cell accompanied by an increase in affinity of the

empty receptor. The net result is normal insulin binding at basal insulin concentrations but decreased binding at high insulin concentrations. The decrease in receptor concentration is proportional to the increase in basal insulin, while the decrease in concentration, increases in affinity, and magnitude of insulin resistance are all proportional to the degree of elevation in GH. It is not yet known whether the insulin resistance seen in acromegaly is due to direct stimulation by GH of insulin secretion leading to insulin-induced binding changes, by GH-induced inhibition of glucose utilization at a site distal to the receptor leading to secondary hyperinsulinemia and resultant changes in binding, or to a direct effect of GH on the insulin receptor.

Elevations in free fatty acids (FFA) are not commonly seen in acromegaly (55,57,58,62), and glucose administration is as effective in suppressing FFA levels in acromegalic patients as in controls (55,57). However, studies of forearm FFA metabolism show an increase in output of FFA in the basal state in patients with acromegaly (64-65). Interestingly, insulin is as effective in suppressing FFA output in acromegalic patients as it is in controls, although it is less effective in increasing glucose uptake. This suggests that FFA levels are normal in acromegaly because elevated plasma insulin levels compensate for the effects of GH on FFA release.

Lipid Metabolism

Rats

GH has been shown to exert both insulin-like and anti-

insulin effects on lipid metabolism when administered acutely. In the rat, this has been studied mainly in hypophysectomized or pancreactomized animals using supraphysiologic doses of GH. The insulin-like action consists of an early decrease in FFA levels. This can be demonstrated both in vivo and in vitro. In vitro studies of rat adipose tissue show inhibition of basal lipolysis and suppression of epinephrine-induced lipolysis (67-68). In vivo, a fall in serum FFA levels is seen 30 to 60 minutes after injection of GH (69-71).

If studies last longer than one hour, a pronounced lipolytic effect of GH is uncovered. By one hour after GH injection, FFA levels begin to rise and continue to increase for at least 5 hours (70). Three and one-half hours after injection of 50 μg GH, fatty acid synthesis from glucose increases, while fatty acid uptake by adipose tissues decreases (69). In pancreactomized hypophysectomized rats, ketones and FFA levels both rise (72). In vitro assays have found increased lipolysis by two hours after administration of GH (73). Dexamethasone potentiates the lipolytic effects of GH both in vivo and in vitro (72-74). These late lipolytic effects can be abolished by administration of inhibitors of protein synthesis (e.g. cycloheximide) and RNA synthesis (e.g. Actinomycin D) within the first two hours after exposure to GH (69,73,75). These inhibitors also prolong the early anti-lipolytic effects of GH (76).

Studies examining the effects of GH on hepatic lipid metabolism have yielded conflicting results. Lotspeich &

Peterson (77) injected rats with 1 mg of GH per day for four days, then gave 5 mg of GH/100 gm body weight on the fifth day and sacrificed the rats 2 hours later. Isolated liver slices showed greatly increased synthesis of acetoacetate and increased liver fat. Penhos et al. (78) perfused rat livers with 0.5 mg/ml of GH for 90 minutes and found increased hepatic uptake of triglycerides and FFA but no increase in ketone production. Chernick et al. (72) perfused rat livers with 5 μg/ml GH for 90 minutes and saw no increase in hepatic uptake of FFA or ketone production. The differences between these studies probably reflect the variations in dose of GH and duration of exposure. The study using the most physiologic dose showed no effect of GH on either hepatic uptake of FFA or hepatic ketone production (72).

Dogs

Data in dogs are also consistent with an early hypolipodemic action of GH, As was the case in rats, these studies used pharmacologic quantities of GH. An early decline in FFA levels after injection with GH is followed by a 4-fold rise in FFA and glycerol levels within three hours as well as an increase in the turnover of FFA and in the percentage of total respiratory CO_2 derived from FFA (79). Of interest is the observation that the rise in FFA levels only lasts for five to ten days. After that, FFA levels return to baseline despite the continued administration of GH (80-82). The return to baseline is not due to depletion of fat stores since norepinephrine stimulated lipolysis can still occur (79). It is also not due to increases in

plasma insulin since insulin levels increase several days before the decline in FFA levels is seen (79).

Humans

In adult humans, GH exerts effects similar to those induced in animals. There have been several reports of an early decrease in FFA levels following administration of large doses of GH. For example, 4 mg GH intravenously causes a 21 per cent fall in plasma FFAs at one hour (83), while 10 mg GH intravenously causes a 33 percent decline in plasma FFA levels by 45 minutes due to a decrease in output (84).

Increases in plasma FFA levels are seen 2 to 4 hours after injection of GH. Several groups have found approximately a 100 percent increase in FFA levels after injection of 4 to 8 mg of GH (80,84-87). GH-induced increases in FFA levels are inhibited by the administration of glucose (80) and by inhibitors of lipolysis such as nicotinamide (87) and 5-methylpyrazole-3-carboxylic acid (86). Insulin is as effective in suppressing lipolysis in subjects injected with GH 4 hours before insulin injection or in subjects infused simultaneously with insulin and GH as it is in control subjects (64,84).

The effect of GH on peripheral uptake and output of FFA has been examined in studies where GH was infused into the brachial artery of normal volunteers. Changes in the arterial minus deep venous FFA concentrations were taken as representative of muscle metabolism and changes in arterial minus superficial venous FFA concentrations were taken as representative of adipose

tissue metabolism. When arterial GH concentrations of 300 ng/ml were achieved, an immediate increase in FFA uptake by muscle was seen followed by a sustained increase in FFA output from adipose tissue beginning at forty minutes (64,88). When lower concentration of GH were infused (28.4 ng/ml to 200 ng/ml) however, no effect of GH on muscle or adipose tissue lipid metabolism was seen during the 90 minute duration of the study (83). The discrepancy between these studies may be due to differences in the dose or duration of exposure to GH.

The effect of GH on ketogenesis in humans has been studied only in insulin dependent diabetics (89). In this experiment, diabetic subjects were divided into 3 groups. One group was given 1 mg of GH 12 hours before the study. The second group was infused with 4 µg/kg GH for 1 hour at the start of the study. A third group served as controls. All 3 groups received heparin in order to elevate plasma FFA levels so that the ketogenic effects of GH could be examined independently of its lipolytic effect. Similar FFA levels were achieved in all three groups but the group treated with 1 mg GH 12 hours earlier had significantly greater increases in ketone bodies. The authors concluded that GH exerts ketogenic activity that is delayed at least 60 minutes in onset. However, it is difficult to ascribe this effect to GH, since GH levels had returned to baseline well before the heparin injection was given. Also, the group that had increased ketogenesis had significantly lower insulin levels. Unless GH itself caused the decreased insulin

levels, it is unlikely that GH was responsible for the increased ketogenesis.

Another study examined the role of basal GH levels in insulin-dependent diabetics (90). In this study, somatostatin was given to suppress glucagon and GH secretion. When GH and insulin were infused along with the somatostatin, no effects were seen. However, when GH was infused to achieve a plasma concentration of 6 ng/ml in the absence of insulin, levels of FFA, glycerol and β-hydroxybutyrate began to increase by 1 hour and were significantly higher than controls at 4 to 6 hours. The authors concluded that basal levels of GH can augment lipolysis and ketogenesis only in the absence of insulin.

No study has examined the effects of physiologic increment in GH, such as those seen during various types of stress, on lipid metabolism in normal subjects.

Carbohydrate Metabolism

Rats

The dichotomy between the early and late effects of GH on lipid metabolism are also apparent in regard to glucose metabolism. In the normal and hypophysectomized rat, injection of GH leads to a reduction in blood sugar within 20 minutes (71,77,91). In vitro incubation of rat muscle, adipose or kidney tissue with GH results in an increase in glucose uptake and oxidation within 20 to 60 minutes (68,91-93). These early effects can be duplicated by incubation of tissue with high concentrations of glucose suggesting that they might be due to an increase in cellular uptake of glucose (93). Increased

cellular uptake of glucose could be mediated by changes in insulin secretion or degradation or in the tissue response to insulin. The importance of insulin in the acute hypoglycemic action of GH is supported by the observation that no acute hypoglycemic effect is seen after GH injection into insulin-deplete diabetic rats (94). Although plasma insulin levels are not elevated within the first hour after exposure to GH, insulin degradation has been reported to transiently decrease (91) and insulin release from the pancreas of hypophysectomized rats (95) and normal rats (96) has been reported to increase.

Exposure to GH for longer than 1 to 2 hours causes hyperglycemia and decreased glucose utilization in the rat. Three hours after exposure to GH, it is possible to show decreased glucose utilization in adipose tissue (69). Four days of treatment with GH and dexamethasone result in glycosuria in partially pancreatomized rats (98). Twelve days of GH injection cause increases in blood glucose and decreased glucose uptake in vitro in hypophysectomized rats (99). These later hyperglycemic effects can be prevented by exposure to inhibitors of protein and RNA synthesis. These also prolong the early hypoglycemic actions of GH (93).

Prolonged exposure to GH in rats has been reported to cause hyperinsulinemia and insulin resistance (100). Rats implanted with a GH-secreting tumor have a 5-fold increase in fasting insulin levels, increased pancreatic islet volume and insulin content and increased glucose stimulated insulin secretion in

vitro. This is accompanied by decreased utilization of glucose by diaphragm and adipose tissue in vitro (100). Conversely, islets taken from hypophysectomized rats have a decreased insulin content and release which returns towards normal after 3 days of treatment with GH (101). Injection of 1 mg GH twice a day for 5 days into normal rats results in a 2 fold increase in plasma insulin levels and a 10 mg/dl increase in blood sugar (102). This is accompanied by a decrease in the concentration of insulin receptors on hepatic membranes and an increase in the affinity of the empty receptor (102), changes similar to these seen in monocytes of patients with acromegaly (66).

Dogs

In the dog GH causes transient hypoglycemia in hypophysectomized animals but not in normal dogs. This is accompanied by an increase in glucose uptake (79). Whether insulin is necessary for the hypoglycemic effect in dogs is not clear, since, in acutely pancreactomized dogs, GH also causes transient hypoglycemia (103). This is the opposite of the situation in the rat. The differences may be due to the presence of residual insulin in the acutely pancreactomized dogs.

More prolonged exposure to GH in both normal and hypophysectomized dogs causes increases in blood glucose and insulin (79, 82,104-105). The hyperglycemic reaction is dependent upon the nutritional status of the dog. Dogs that have been fasted for four to five days before receiving GH maintain a normal blood glucose, but this is in the presence of marked hyperinsulinemia and insulin resistance (79). The hyperglycemia is

due to a decrease in peripheral glucose clearance and an increase in the hepatic release of glucose (79,82,105).

Humans

In normal humans, pharmacologic doses of GH have been shown to induce a transient decrease in blood glucose of approximately 10 mg/dl within the first hour after exposure to GH (83-84, 106-107). This has been attributed to a decrease in glucose output (84,107). Very early after GH administration (10 minutes), it can be shown that glucose utilization as measured by the intravenous glucose tolerance test (IVGTT) increases by 30 percent (62,85).

Longer term administration of pharmacologic doses of GH causes increases in blood glucose only in hypopituitary patients (53) and obese patients during a period of starvation (108). However, results of IVGTT in normal subjects reveal a 50 per cent decline in glucose utilization 2 to 5 hours after GH injection (62,85-87). Glucose-stimulated insulin release has been reported to increase (62,106), to remain unchanged (87), and to decrease (109) following GH administration.

To evaluate the effect of acute administration of GH on peripheral glucose metabolism, GH was infused into the brachial artery of normal subjects while arteriovenous differences in glucose concentration were measured. When pharmacologic doses of GH were infused, an immediate 50 percent reduction in glucose uptake by muscle and adipose tissue was seen (64,88). When more physiologic doses were infused for 30 minutes, a 30 percent decrease

in glucose uptake into muscle tissue was seen (83). In neither study was any early insulin-like action observed.

There is some evidence that short term GH infusions can lead to a state of insulin resistance. Infusion of GH into the brachial artery along with insulin blocks the effects of insulin on glucose uptake (64, 65). Giving an insulin infusion four hours after 5 mg of GH results in attenuation of insulin induced increases in glucose uptake (84). Amino acid-stimulated insulin release is 2-fold greater in GH treated subjects than in controls but does not cause any change in blood sugar (110). Finally, obese subjects in the fed state given GH have increased insulin levels but normal blood sugars (108).

Most of the previously cited studies were done using pharmacologic doses of GH. An attempt to examine the consequences of physiologic fluctuations in GH levels was made by Yalow et al. (111). They provoked increases in plasma GH by giving a 100 gram oral glucose load to normal subjects. Plasma GH began to rise, in response to falling blood glucose levels, 3 to 4 hours after the oral glucose load was given and peaked at about 13 ng/ml at 4 to 5 hours. Six hours after the original glucose load, a repeat 100 gm oral GTT was performed. In those subjects who had shown an elevation in GH in response to the first glucose load, the second GTT showed significantly decreased glucose tolerance and markedly increased glucose-stimulated insulin release. Those subjects who did not augment their GH secretion in response to the original glucose load had no deterioration of glucose

tolerance during the second test. This seems to indicate that physiologic elevations in plasma GH levels cause a deterioration in glucose tolerance and increased insulin secretion. However, when the oral GTT was repeated 4 hours after the initial glucose load, at the time of the peak GH response, glucose tolerance was only slightly impaired and glucose-stimulated insulin secretion was not increased. Finally, repeating the oral GTT 3 hours after the initial glucose load resulted in improved glucose tolerance and decreased insulin secretion. The major difficulty in interpreting this set of experiments is that many factors other than GH are responsive to changes in glucose levels. It is likely that fluctuations in the plasma levels of epinephrine, norepinephrine, cortisol and glucagon occurred (112) and also influenced the results.

In another series of studies, GH was infused into healthy subjects for 30 minutes at different doses (109,113-114). The two lowest doses, 5 and 10 μg/kg, resulted in post-infusion GH concentrations of 20 and 48 ng/ml, levels simulating those seen during stress. Blood glucose was found to decrease slightly but significantly only in the group receiving 5 μg/kg GH infusions, not in the group that received 10 μg/kg GH infusions. However, both groups showed significant decreases in basal plasma insulin that were apparent by 15 minutes after the start of the GH infusion and lasted up to 60 minutes after the infusion ended (109). There was no correlation between the timing and the magnitude of the changes in insulin and glucose. When glucose utilization was

estimated by the IVGTT, it was found that glucose tolerance measured at the end of a 30 minute 10 μg/kg GH infusion had deteriorated significantly (113). However, glucose tolerance continued to deteriorate even further over the five hours following the end of the infusion despite the fact that GH levels had returned to baseline by 1 hour after the end of the GH infusion (109,113). Glucose-stimulated insulin secretion was unaffected by infusion of physiologic doses of GH in this study (113), but in a later study which divided the subjects into 3 groups, mild diabetics, low and high insulin responders, glucose-stimulated insulin secretion was found to decrease in the diabetic and low insulin responders (114). Glucose tolerance, however, decreased in all three groups. There are several difficulties in interpreting these studies. One problem is that GH concentrations were not kept constant during the study period making it unclear whether decreased glucose tolerance and suppression of insulin release were in fact due to the GH administered 5 hours earlier. Another difficulty is explaining the suppression of insulin levels since most of the data available in humans and animals support the hypothesis that GH leads to increased levels of insulin. There is no information available on the effects of a sustained increase of GH to levels seen normally during stress on insulin secretion, glucose levels, and glucose tolerance.

Protein Metabolism

Animals

It has been known that GH influences protein metabolism since the early studies of Evans and Long (115) and Teal and Cushing (116) demonstrated increased growth of normal rats

and dogs treated with pituitary extracts. Later it was discovered that treatment of normal or hypophysectomized animals with pharmacological doses of GH caused a decrease in both blood amino nitrogen levels and urinary nitrogen excretion that is apparent by one hour after exposure to GH (117, 118) and lasts during several weeks of GH therapy. The effects of GH on protein metabolism have recently been reviewed (119).

One action of GH is to increase the rate of uptake of amino acids into skeletal muscle, cardiac muscle and liver. This occurs after a 30 to 60 minute lag period and disappears after 3 to 4 hours (119). Administration of inhibitors of protein synthesis such as puromycin and cycloheximide or inhibitors of RNA synthesis such as Actinomycin D prolong the stimulatory action of GH on amino acid uptake (119). In vitro studies using radioactively labelled amino acids have shown that GH increase the uptake of glycine, alanine, serine, threonine,histidine, proline, tryptophan, asparagine and glutamine but does not effect the rate of uptake of valine, phenylalanine, leucine, methionine, tyrosine, lysine, arginine, glutamate or aspartic acid (120).

It has been suggested that augmentation of amino acid transport is mediated by GH-induced increases in plasma insulin (119). This is unlikely since insulin levels do not increase until after the peak increase in amino acid uptake has occurred. In addition, increased uptake occurs after exposure to GH in

vitro as well as in vivo (119). Finally, inhibition of phosphodiesterase with theophylline or caffiene blocks GH induced changes in animo acid transport but does not affect insulin-induced transport changes (119).

GH also acts to increase the rate of protein synthesis in muscle and liver (119). This effect occurs after a 30 minute lag period but,unlike the increase in amino acid uptake, persists for at least several days. In vitro studies using labelled amino acids have shown a 30 to 50 percent increase in incorporation of glycine, alanine, serine, proline, histidine, threonine, methionine, tryptophan, tyrosine, leucine, valine, phenylalanine, lysine and arginine into protein (120). Thus different amino acids are involved in transport and synthetic effects of GH. These two processes were also shown to be separate in a study in which amino acid uptake was blocked by removal of Na from the incubation medium (121). Under these conditions, stimulation of protein synthesis is unaffected. GH causes increased protein synthesis,at least in part, by enhancing the catalytic activity of ribosomes (122) and increasing the number of ribosomes (123). The changes in ribosomal activity reach a maximum at 18 to 24 hours after exposure to GH.

Salmon and Duvale (124) suggested that somatomedins, not GH, are the ultimate stimulus of increased protein synthesis in muscle. However,this was disputed by Kyosto and Nutting (125) who found that while protein synthesis began to increase after 30 minutes, somatomedin levels in blood did not begin to increase for 6 to 24 hours after administration of GH.

This does not exclude the possibility that somatomedins mediate the prolonged duration of the increase in protein synthesis.

The above studies on amino acid uptake and protein synthesis have all been performed on hypophysectomized animals. It has not been possible to demonstrate any changes in uptake or synthesis in normal adult rats even with administration of pharmacologic quantities of GH. Recently, enhancement of both these processes has been found after in vivo and in vitro GH treatment in young (17 to 18 day) normal rats (126-128). The characteristics of enhanced amino acid transport were similar to those observed in hypophysectomized animals,whereas only a transient increase in protein synthesis was found.

Humans

The effects of GH on protein metabolism in human subjects has not been studied as extensively as in animals. It is known that treatment of hypopituitary patients and normal subject with large doses of GH results in decreased urinary nitrogen excretion, decreased plasma amino nitrogen and a positive nitrogen balance (129-131). Normal subjects are less sensitive than hypopituitary patients to GH administration (132). The response of obese subjects treated with large doses of GH for 5 days is dependent upon their nutritional status (108). Patients in the fed state respond to GH by decreasing urinary excretion of urea and ammonia with a resultant positive nitrogen balance. After prolonged starvation of obese subjects, GH causes a 50 percent decrease in urea excretion but this is balanced by a 50 percent increase in ammonia excretion so that there is no net change in nitrogen balance. The authors

concluded from this that GH does act to decrease protein catabolism, but, in starvation, the lipolytic and ketogenic effects of GH result in increased urinary acid secretion in the form of ammonia and oppose the protein-sparing effects of GH.

The effect of GH on specific amino acid levels in blood has been examined in normal patients after injection of 4 mg GH intravenously (133). Leucine decreased by 50 percent 30 minutes after GH injection and levels remained depressed for the 4 hour duration of the study. Methionine, tyrosine, and alanine levels fell significantly by 30 minutes but returned to baseline by 2 hours. Decreases in histidine were not seen until 2 hours after GH exposure. Glycine levels actually rose at 30 and 60 minutes and then returned to baseline. No other amino acids were studied. The acute effects of physiologic increments in GH on amino acid levels in normal subjects are not known.

MATERIALS AND METHODS

Subjects

Studies were performed on 8 healthy subjects, 5 male and 3 female, with a mean age of 26.4 ± 4.2 years. All were within 15 percent of ideal body weight (Metropolitan Life Insurance Tables, 1959). Details are in Table 1. Subjects consumed weight-maintaining diets containing at least 200 gm of carbohydrate and were taking no drugs. All had negative primary family histories for diabetes and none had an elevated fasting plasma glucose. The subjects were informed of the nature, purpose and possible risks of the study before their written, voluntary consent to participate was obtained. Prior approval for these investigations was obtained from the Yale Human Investigations Committee.

Experimental Design

Studies were begun in the morning after a 12 to 15 hour overnight fast. A polyethylene catheter was inserted into an antecubital vein for blood sampling and in the contralateral vein for administration of human growth hormone (HGH). Two experimental protocols were employed in each subject. In the first, HGH, at a dose of 3 μg/kg/hr, was infused for 8 hours at a flow rate of 14.4 ml/hr by a Yale Digital Infusion Pump. HGH (obtained from the National Pituitary Agency) was prepared by first dissolving it in 10 ml of distilled water and then diluting it into a larger volume of 0.9% NaCl. In the second study, physiologic saline, rather than HGH, was infused for 8 hours under identical conditions. After either HGH or saline had been infused for 5 hours, a 3 hour 100 gm

oral glucose tolerance was begun (Dexerol). The first four sets of studies were performed in random order. In the second four sets of experiments, the saline infusions were performed first because of concern about possible long term effects of HGH infusion.

Some subjects also participated in a third study in which 0.9% NaCl or 3 μg/kg/hr HGH were infused for 5 hours for measurement of insulin binding to monocytes and erythrocytes. Individual studies were separated by a minimum of 48 hours and a maximum of 14 days. No subject experienced any unpleasant side effects.

SAMPLING PROCEDURES

Blood for glucose measurement was drawn at times t=-15,0,30,60,120, 180 and 240 minutes and at 15-30 minute intervals thereafter. Samples were immediately centrifuged and the plasma used for assay of glucose.

Samples for plasma immunoreactive insulin were collected in heparinized tubes at the same time intervals as the glucose samples were collected.

3ml of blood was added to tubes containing 0.3ml Trasylol and 10.5 mg EDTA for determination of immunoreactive glucagon. Samples were obtained at t=-15,0 and 60 minutes and every 60 minutes thereafter up to 480 minutes.

Samples for measurement of FFA were collected in heparinized tubes at t=-15,0 and 60 minutes and every 60 minutes thereafter up to 300 minutes.

Equal volumes of blood were mixed with 6.0% perchloric acid for determination of acetoacetate and β-hydroxybutyrate. Samples were obtained at t=-15,0 and 60 minutes and every 60 minutes thereafter up to 300 minutes.

Samples for measurement of plasma amino acids were obtained in heparinized tubes at the same time intervals as the ketone samples were obtained. The plasma was deproteinized with 10% sulfasalicylic acid.

Plasma for measurement of HGH was obtained at t=-15,0,60,180 and 300 minutes and at 30 to 60 minute intervals thereafter.

At t=0 and 240 minutes blood was collected in heparinized tubes for measurement of insulin binding to monocytes and erythrocytes.

Glucose and insulin binding assays were performed on the day of the study. All other samples were stored at -20°C until used for analyses.

CHEMICAL ANALYSES

Plasma glucose was assayed by the glucose oxidase method (134) on a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California).

Plasma immunoreactive insulin was determined by radioimmunoassay using talc to separate bound from free insulin (135).

Plasma immunoreactive glucagon was measured by radioimmunoassay using the Unger 30K antibody (136).

Plasma growth hormone was measured by radioimmunoassay (137) by the Yale New Haven Hospital Clinical Immunology Laboratory.

β-hydroxybutyrate and acetoacetate concentrations in blood were determined by the method of Williamson, Mellanby, and Krebs (138).

FFA levels were determined enzymatically by a modification of the method of Dole (139).

Plasma amino acids were assayed by automatic ion-exchange chromatography (140). This method measures neutral and acidic but not basic amino acids.

Insulin binding to monocytes was determined using ^{125}I-monoiodoinsulin (141). Monocytes were isolated from 50 ml of whole blood and incubated for 3 hours with 0.2 ng/ml ^{125}I-insulin in the presence and absence of unlabelled porcine insulin. The specific binding of ^{125}I-insulin was calculated by subtracting nonspecific binding (^{125}I-insulin bound in the presence of unlabelled insulin) from total ^{125}I-insulin binding (^{125}I-insulin bound in the absence of unlabelled insulin). Total binding capacity was calculated by Scatchard analysis (142).

Insulin binding to erythrocytes was determined by the method of Gambhir, Archer, and Carter (143). Erythrocytes were isolated from 10 ml of blood using a Ficoll-Hypaque gradient and incubated for 2.5 hours with ^{125}I-insulin in the presence and absence of unlabelled insulin. RBC-bound insulin was separated from free insulin using dibutyl phthalate. Specific binding and total receptor capcity were calculated as in the monocyte binding studies.

STATISTICAL METHODS

All values represent the mean of duplicate determinations of each sample. Data is given as the mean ± standard error. Analyses was performed using the Students T-test. The paired t-test was used to compare results from GH infusion with results from the control saline infusion (144).

TABLE I

Subject	Age	Sex	Height (cm)	Weight (kg)	Percent Normal Weight
V.P.	18	M	180	61.4	85
N.R.	38	F	157	53.6	110
D.F.	35	M	183	79.5	107
L.S.	22	F	170	62.3	107
T.B.	18	M	175	65.9	96
T.W.	34	M	175	72.7	106
C.C.	23	F	180	72.7	108
B.O.	23	M	188	85.0	107

RESULTS

Growth Hormone Levels

Mean preinfusion GH levels were similar in both the saline and GH infusion studies (saline, 2.2±0.8 ng/ml vs GH, 1.8±0.3 ng/ml, $p>0.05$). As can be seen in Figure 1, GH levels increased rapidly during the infusion of GH, reaching a stable plateau within 2 hours. The mean plateau value was 35.2±4.4 ng/ml. Individual preinfusion and plateau GH concentrations are shown in Table 2.

Effect of GH on Basal Substrates

The first 5 hours of these studies, before the start of the oral glucose tolerance test, is considered the basal period. Mean basal glucose concentrations during GH and saline infusion are given in Table 3. Glucose levels remained nearly constant throughout the basal period and there was no significant difference between the GH and corresponding saline control glucose levels.

Figure 2 demonstrates the changes in FFA and ketone levels that occurred during the infusion of GH. FFA levels (491±43μM before GH infusion) increased by 76% in the GH group (to 866±74μM) whereas they increased by only 25% (to 576±66μM) in the fasting saline control group. The differences between the corresponding saline and GH FFA levels were statistically significant after 180 minutes ($p<0.05$-0.005). Similiarly, blood ketone levels (0.110±0.08mM before GH infusion) increased 86% during the GH infusions and were significanly higher than the corresponding saline control values at 180 minutes ($p<0.05$) and 300 minutes ($p<0.01$).

In 3 subjects, 11 different amino acids were measured during the

infusion of GH (Table 4). Since in these studies only plasma alanine appeared to change substantially, we elected to further evaluate this question in all subjects by measuring alanine using a short column technique. The latter studies also provided data on glycine, valine, leucine, and isoleucine which is included in Table 5. These data show that alanine decreased by 22% during the GH infusion and was significantly lower than preinfusion values at 240 minutes ($p<0.001$) and 300 minutes ($p<0.005$). In addition, there was a small decline in plasma glycine (8%, $p<0.05$), whereas the concentrations of the branch chained amino acids(valine, leucine, and isoleucine) remained unchanged. There were no significant changes in amino acid concentrations during the control saline infusions.

Effect of GH on Basal Hormone Levels

As shown in Table 3, plasma insulin levels were 35% greater in the GH-infused subjects than in the saline controls at 5 hours. Figure 3 demonstrates 0 and 5 hour plasma insulin levels in the saline and GH groups. It is apparent that insulin levels tended to increase during the GH infusions whereas they tended to decrease during the saline infusions. The difference between the two groups became significant by 5 hours (GH, 16.3±1.1 μU/ml vs saline, 12.1±0.9 μU/ml, $p<0.005$). In contrast, basal plasma glucagon levels decreased slightly in control and GH studies and never differed significantly between groups (Table 3).

Effect of GH on the Response to a Glucose Load

The GH response to a 100 gram oral glucose load is shown in Figure 1. As expected (5,13), mean GH levels tended to fall in the

saline control subjects from 4.5±1.7 ng/ml at 300 minutes to 1.87 ±0.5 ng/ml by 60 minutes after ingestion of glucose. However, these changes were not statistically significant. After 60 minutes, GH levels returned to values seen prior to glucose. In the GH-infused subjects, plasma GH remained unchanged.

Figure 4 demonstrates the mean glucose and insulin responses to the 100 gram oral glucose load. GH treatment resulted in marked deterioration in glucose tolerance with plasma glucose levels 30 to 50 mg/dl above the corresponding saline control values from 90 minutes after glucose administration until the end of the study (90 min., 170±11 mg/dl vs 127±11 mg/dl, $p<0.005$; 120 min., 162±12 mg/dl vs 121±7 mg/dl, $p<0.01$; 180 min., 124±13 mg/dl vs 85±6 mg/dl, $p<0.05$). The incremental area under the glucose curve during the glucose tolerance test was increased by 85% in the GH-treated subjects as compared to the saline controls (11078±1571 mg/dl/180 min. vs 5885 ±1069 mg/dl/180 min., $p<0.005$).

The decreased glucose tolerance occurred despite an increase in insulin secretion. Mean peak insulin levels were 2-fold higher in GH-treated subjects (168±34 μU/ml vs 86±18 μU/ml, $p<0.005$) and the incremental area under the insulin curve was 82% greater than in the saline control (18404±3306 μU/ml/180 min. vs 10145±2272 μU/ml/180 min., $p<0.005$). Insulin levels in the GH-infused subjects were significantly higher than in the saline controls at 60 minutes (108±19 μU/ml vs 86±18 μU/ml, $p<0.005$), 120 minutes (168±34 μU/ml vs 80±22 μU/ml, $p<0.005$), and 180 minutes (128±28 μU/ml vs 41±7 μU/ml, $p<0.02$) after glucose ingestion. The time course of the insulin response also differed between groups. During the saline infusions, peak insulin

levels occurred at 60 minutes whereas during the GH infusions, insulin levels did not peak until 120 minutes after glucose administration.

Figure 5 compares individual 120 minute glucose and insulin levels in the GH and saline groups. It shows that 2 of 8 subjects (open circles) did not have significantly different glucose levels in the GH and saline infusions but that all subjects had elevated insulin responses after GH infusion. The glucose and insulin responses of the 2 subjects whose glucose tolerance did not deteriorate after GH treatment are described in more detail in Figure 6. Although the total area under their glucose curves remained unchanged, the area under their insulin curves increased by 67% (subject D.F.) and 73% (subject L.S.).

During the glucose tolerance test, plasma glucagon decreased by 30% in the GH-treated subjects and by 12% in the saline controls (Table 6). The differences between the two groups never attained statistical significance.

There was no correlation between the absolute decrease in glucose tolerance and the absolute increase in the insulin response ($R=-0.2$, $p>0.05$) or between the percentage changes in glucose and insulin ($R= 0.3$, $p>0.05$). Nor was there any relationship between the magnitude of the plateau GH elevation and the glucose ($R=-0.07$, $p>0.05$) or insulin ($R=0.2$, $p>0.05$) response to the glucose load.

TABLE 2

Individual Growth Hormone Levels

		0 Minutes	Plateau
HGH	V.P.	2	44
ng/ml	N.R.	1	28
	D.F.	1	40
	L.S.	3	25
	T.B.	1	25
	T.W.	2	35
	C.C.	2	25
	B.O.	1	60
	Mean	1.6	35.2
	SEM	0.25	4.4

FIGURE 1

EFFECT OF GROWTH HORMONE INFUSION ON PLASMA GROWTH HORMONE LEVELS

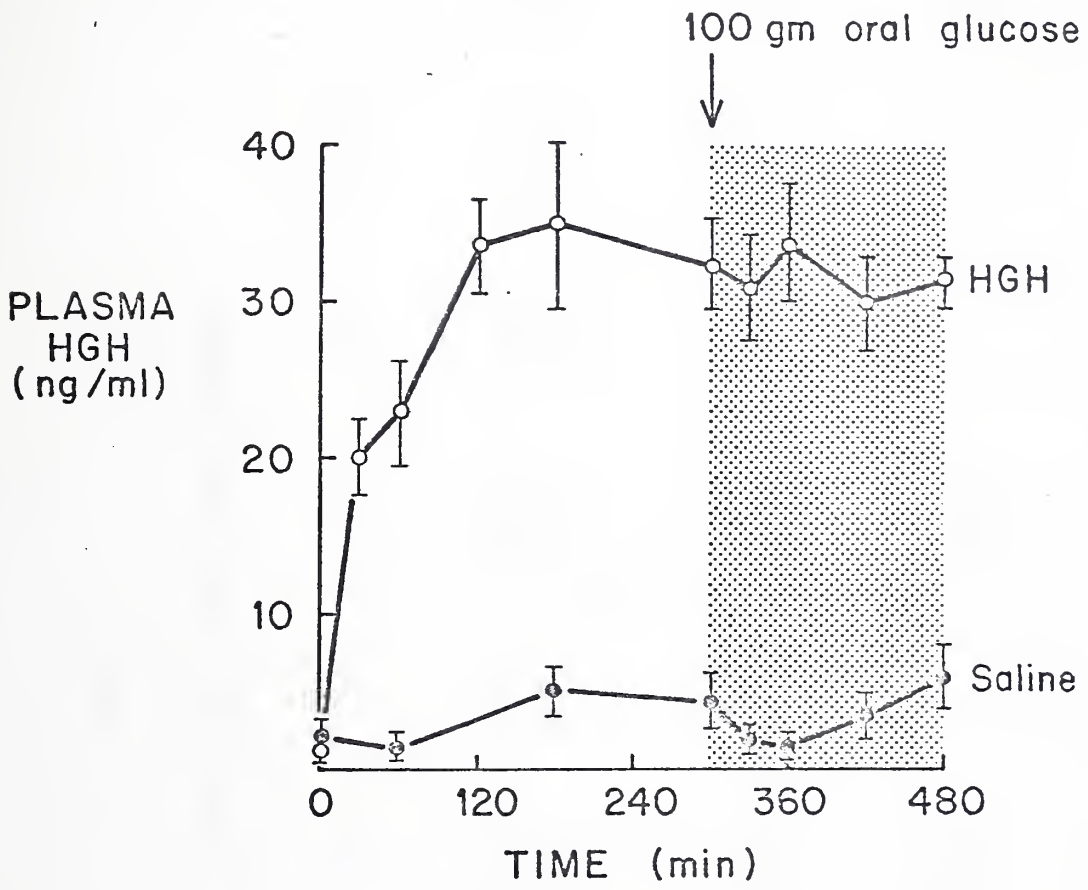


TABLE 3

MEAN BASAL PLASMA GLUCOSE, INSULIN AND GLUCAGON

Minutes	0	30	60	120	180	240	285	300
Glucose (mg/dl)								
HGH	87±3	88±3	86±2	85±2	84±2	86±2	85±2	85±2
Saline	90±3	92±3	89±2	88±3	87±2	86±1	85±1	86±2
Insulin (μU/ml)								
HGH	14.0±0.65	14.7±1.1	12.7±0.9	13.6±1.5	14.1±1.4	14.4±0.8	17.1±1.1	16.3±1.1
Saline	15.7±1.5	15.7±1.3	13.4±1.0	13.3±1.3	13.3±1.3	12.7±1.1	12.7±1.1	12.1±0.9
Glucagon (pg/ml)								
HGH	144±13		133±14		121±17			134±12
Saline	140±15		118±15		119±18			125±20

EFFECT OF GROWTH HORMONE INFUSION ON BASAL LEVELS OF FFA AND KETONES

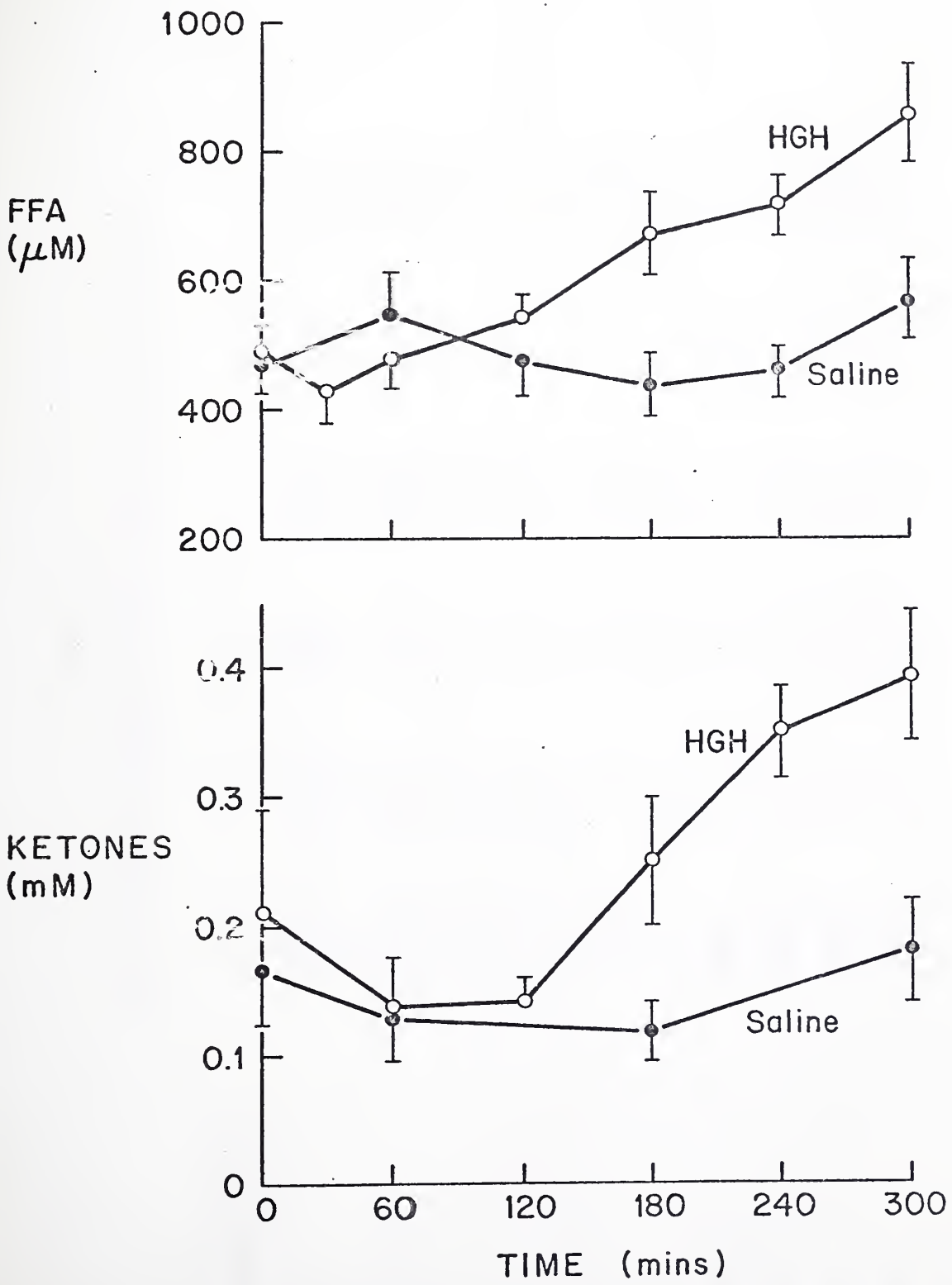


TABLE 4

EFFECT OF GROWTH HORMONE ON BASAL AMINO ACIDS IN 3 SUBJECTS

Plasma Amino Acids

Minutes	0	60	120	180	240	300
	µm	µm	µm	µm	µm	µm
Taurine	39±4	46±5	37±5	39±4	33±2	37±4
Threonine	139±40	151±35	145±32	142±38	131±35	131±31
Serine	107±26	114±21	108±20	103±22	100±23	100±18
Proline	131±60	140±63	125±54	125±59	125±64	117±30
Methionine	26±6	26±5	25±4	25±7	24±8	24±7
Tyrosine	55±14	55±13	52±10	52±15	48±16	48±16
Leucine	113±7	120±4	118±8	125±6	120±10	124±5
Isoleucine	58±2	61±3	58±6	61±1	57±4	58±1
Glycine	248±35	260±24	244±24	238±30	228±26	223±23
Valine	190±10	198±14	197±11	191±9	186±11	189±12
Alanine	345±74	362±74	334±60	312±67	273±61	259±51

TABLE 5

EFFECT OF GROWTH HORMONE ON BASAL AMINO ACIDS

Minutes	0	60	120	180	240	300
	µm	µm	µm	µm	µm	µm
Alanine-Saline	325±29	320±19	319±24	309±21	294±22	302±20
HGH	360±46	363±45	334±42	325±47	292±39*	279±35**
Valine-Saline	238±9	241±12	235±12	231±14	227±11	235±15
HGH	208±13	209±11	196±14	196±12	195±8	198±8
Glycine-Saline	234±25	241±26	241±26	238±27	232±21	242±21
HGH	248±21	255±22	243±18	240±20	233±17	228±17***
Leucine-Saline	97±10	95±7	101±12	95±9	98±11	95±8
HGH	99±7	101±8	99±10	100±9	94±9	101±8
Isoleucine-Saline	44±7	39±3	44±7	36±3	39±6	36±4
HGH	43±5	47±6	45±6	43±6	39±6	40±5

* = $p<0.001$
** = $p<0.005$
*** = $p<0.05$

FIGURE 3

EFFECT OF GROWTH HORMONE AND SALINE ON BASAL INSULIN LEVELS

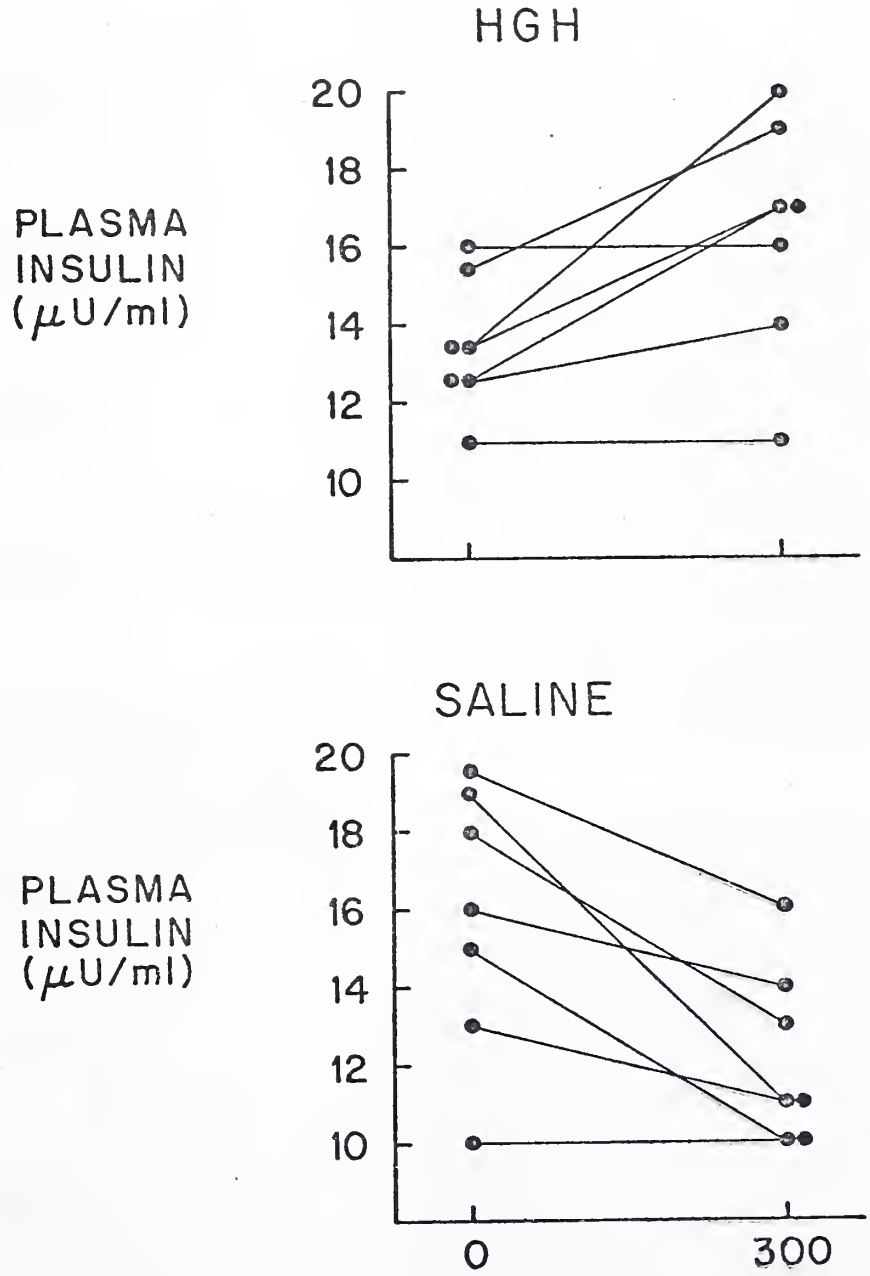
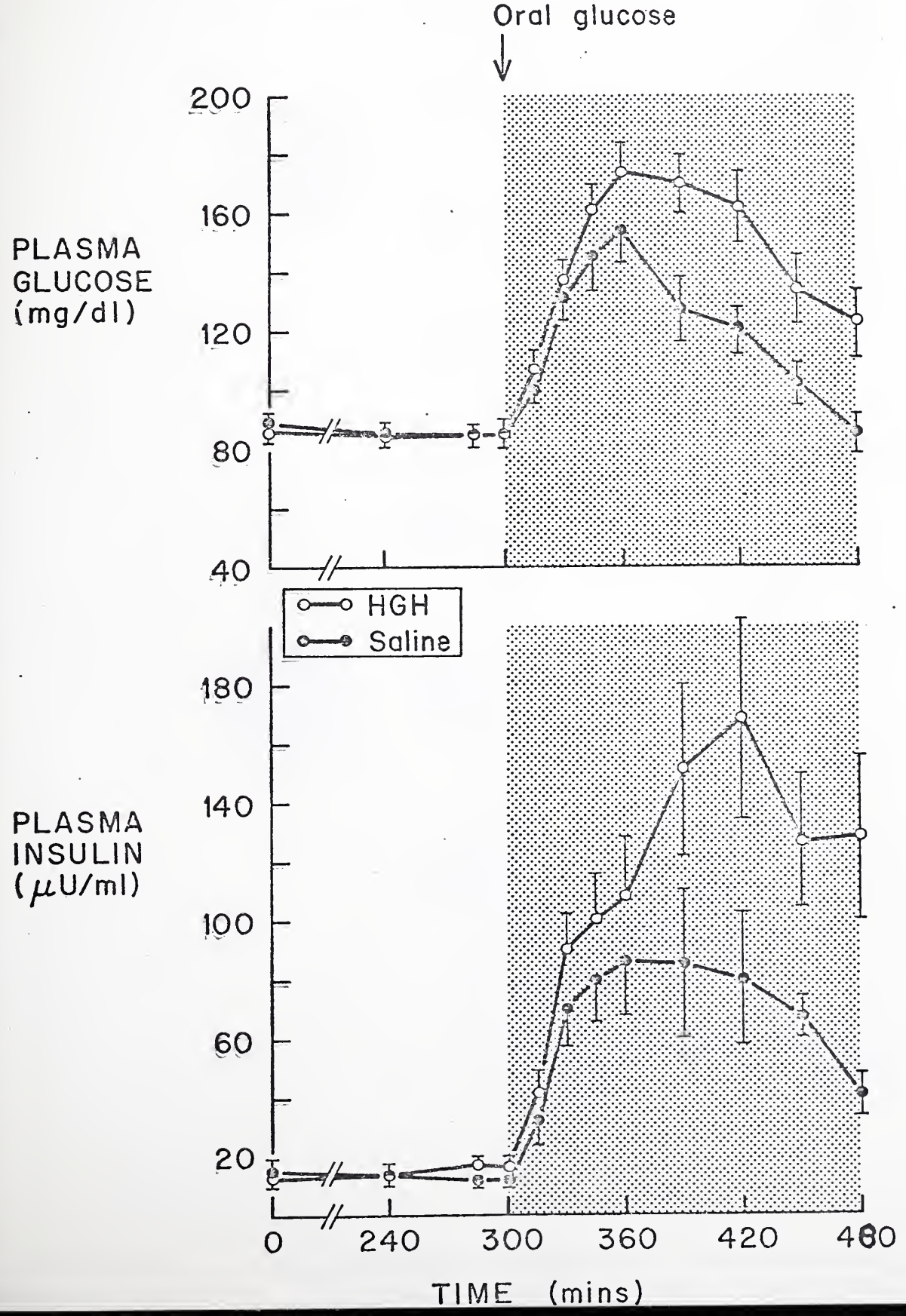


FIGURE 4

EFFECT OF GROWTH HORMONE ON GLUCOSE AND INSULIN RESPONSE TO ORAL GLUCOSE



EFFECT OF GROWTH HORMONE ON PLASMA GLUCOSE AND INSULIN 2 HOURS POST GLUCOSE INGESTION

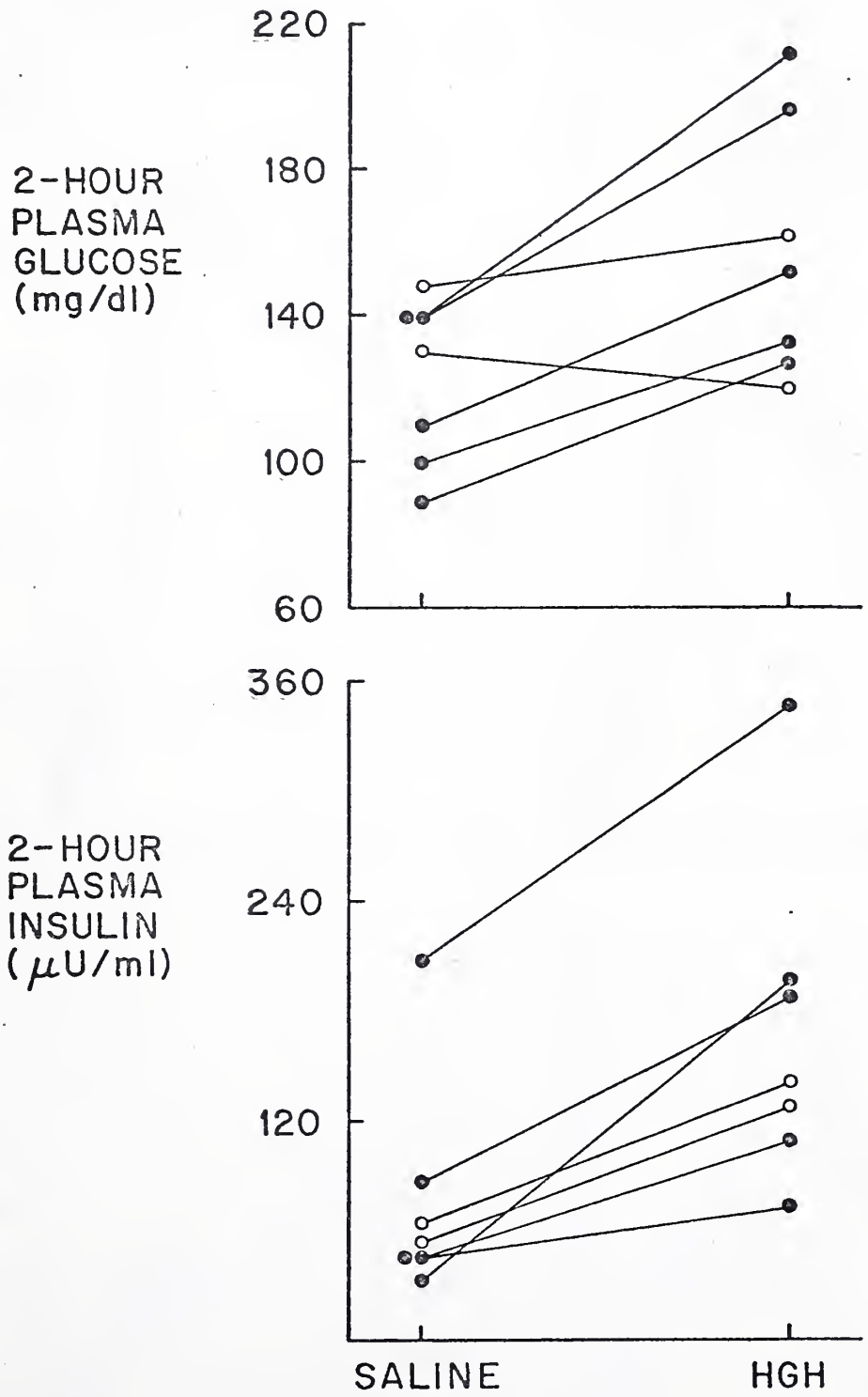


FIGURE 6

INSULIN RESPONSE IN 2 SUBJECTS WITHOUT HGH-INDUCED GLUCOSE INTOLERANCE

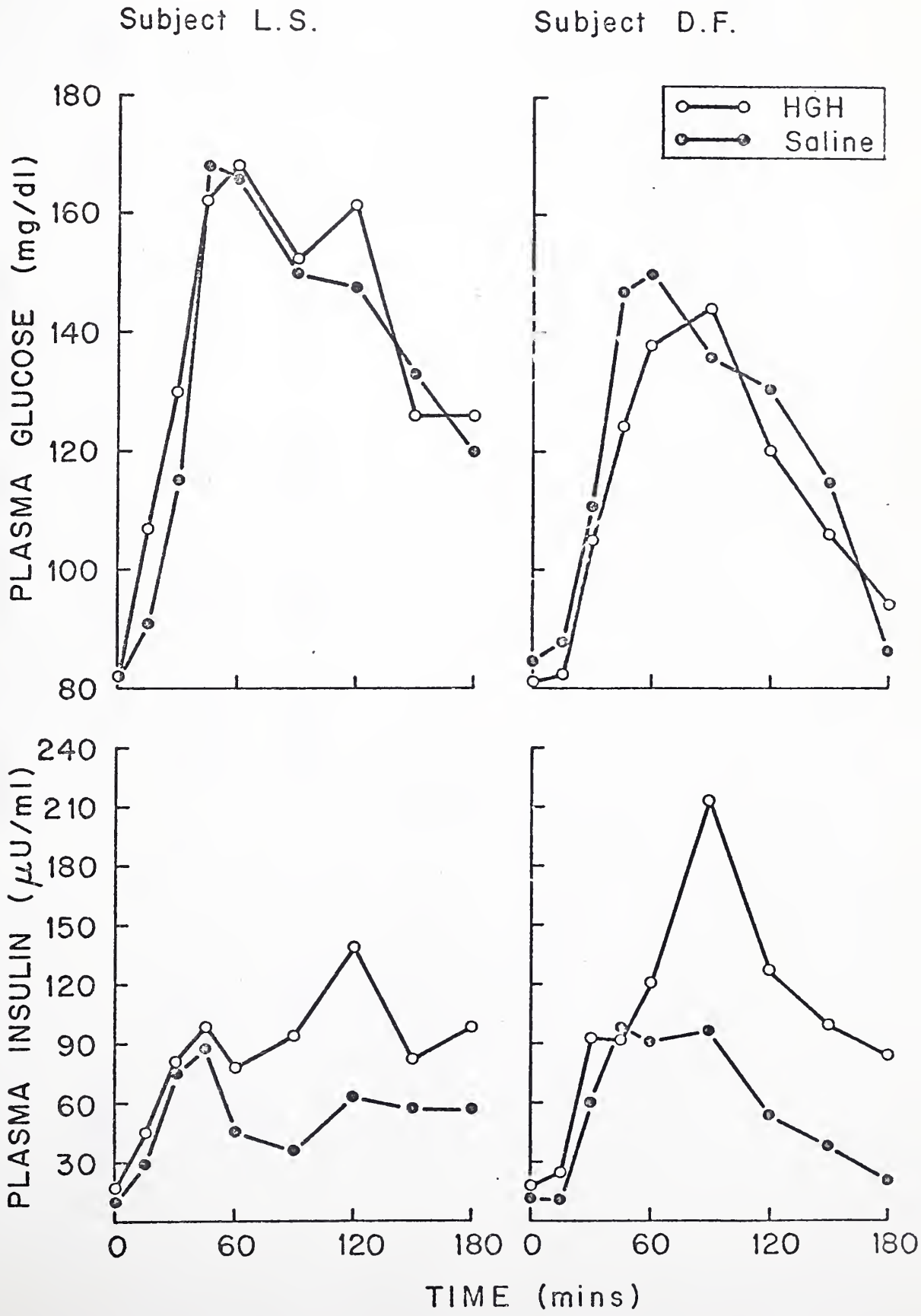


TABLE 6

GLUCAGON LEVELS DURING GLUCOSE TOLERANCE TEST

Minutes		0	15	30	60	120	180
Glucagon pg/ml	HGH	134±12	135±11	113±17	96±13	91±13	93±11
	Saline	125±20	118±9	113±16	114±14	108±15	110±17.5

DISCUSSION

The current data demonstrate that acute physiologic elevations of plasma GH levels, which do not affect basal plasma glucose concentrations, result in marked deterioration of glucose tolerance. In addition, GH infusion causes elevations in basal FFA and ketone levels, in the face of a rise in plasma insulin concentration, as well as a reduction of plasma alanine levels. The magnitude of the elevations in GH achieved during the GH infusions were in the range of those commonly seen during surgery (5,18-22), infection (25-28), exercise (5,15,17), diabetic ketoacidosis (7,29-33), uremia (37-39) and cirrhosis (34-46). Although in some situations only transient GH elevations are observed (17,19), in others, such as severe trauma (145), major surgery (18,22) and diabetic ketoacidosis (29,32), GH often remains elevated for more prolonged periods of time, ranging from 5 to 6 hours (29) to several days (18,22,145). Thus, it is likely that our findings are relevant to the metabolic changes seen during severe stress.

After an overnight fast, plasma glucose concentrations are maintained at a constant level by release of glucose from the liver at a rate equal to the rate of glucose utilization by peripheral tissue (146). Approximately 70% to 75% of hepatic glucose release is derived from gluconeogenesis (146). In this study, plasma glucose concentrations did not differ significantly in the GH-treated subjects as compared to the saline controls. In contrast, several groups have reported a slight decrease in plasma glucose levels 30 to 60 minutes after administration of pharmacologic quantities of GH (83-84, 106-107). The present data support the

view that acute hypoglycemia is a pharmacologic, not a physiologic, action of GH and that physiologic elevations of GH do not have a major effect on basal hepatic glucose output or on basal peripheral glucose utilization.

Despite the maintenance of normal basal glucose levels during the infusion of GH, glucose tolerance was significantly reduced. This is similar to the findings in studies using pharmacologic quantities of GH (62,85-87) and to the findings in acromegaly (55-59) of deterioration in glucose tolerance in many, but not all, subjects. It is of interest in the regard, that in two of our patients, GH had little effect on glucose tolerance, although insulin secretion was enhanced in these individuals.

In normal postabsorptive subjects, a 100 gram oral glucose load is distributed throughout the body as follows: 1) Less than 5% remains in the glucose space after 3 hours, 2) 25% is utilized by noninsulin-dependent tissue, such as brain and renal medulla, to meet ongoing metabolic needs, 3) 15% is utilized by insulin-dependent peripheral adipose and muscle cells, and 4) 55% to 60% is taken up by the liver (146). In addition, hepatic glucose production decreases to 75% to 80% below basal levels (147).

GH could conceivably effect any one of the above steps, although there is no evidence to suggest that it alters the space of glucose distribution or that it effects the utilization of glucose by insulin-independent, glucose-dependent tissue like the brain. Adamson et al.(107) observed that pharmacologic doses of GH actually inhibited splanchnic glucose production by 30% to

40% in fasting subjects and had no effect on the hepatic response to an intravenous glucose load. These findings suggest that the effect of GH to decrease glucose tolerance may not be mediated by an effect on hepatic glucose output. Since the hepatic response to oral and intravenous glucose differ, with significantly less splanchnic glucose uptake after an intravenous glucose load as compared to an oral glucose load (148), these findings do not eliminate the possibility that GH had an effect on hepatic glucose uptake. Finally, evidence from other studies suggests that GH causes decreased peripheral uptake of glucose by muscle and fat tissue (64, 83, 88). Since peripheral uptake by adipose and muscle cells accounts for the disposal of 15% of an oral glucose load, it might explain the decreased glucose tolerance observed in this study.

While earlier studies have reported increases in circulating FFA 2 to 4 hours after administration of pharmacologic doses of GH (80, 84-87), the effect of physiologic GH elevations have not previously been examined. Our findings indicate that the pattern of response is quite similar whether physiologic or pharmacologic doses are given, i.e. FFA begin to increase 2 hours after administration of GH to approximately 100% preinfusion values. In our studies, there was no evidence of an early hypolipademic effect, which supports the hypothesis of Merimee and Rabin (10) that the insulin-like actions of GH previously reported (83-84) are only of pharmacologic, not physiologic, significance. The increase in FFA was clearly not due to hypoinsulinemia since insulin levels actually increased in the GH-infused subjects. Although this study did not address the question of the mechanism of this effect,

results of previous studies, utilizing pharmacologic doses of GH, suggest that GH acts directly on the adipocyte to increase lipolysis in a process that is dependent upon protein and RNA synthesis (69,73,75,86).

With respect to the role of FFA in the glucose intolerance observed in this study, it has been suggested that elevations in circulating FFA levels may reduce glucose uptake by adipose and muscle tissue (149). However, other studies have found that inhibition of GH-induced lipolysis either does not prevent (87) or only partially prevents (86) GH-induced deterioration of glucose tolerance. In this study, FFA levels increased in the 2 subjects who did not exhibit deterioration of glucose tolerance to the same extent as in the 6 subjects who did demonstrate decreased glucose tolerance. It is unlikely, therefore, that elevated FFA levels accounted for the changes in glucose tolerance seen during treatment with GH.

Blood ketone levels also increased during the infusion of GH. This might by secondary to GH-induced FFA elevations leading, in turn, to increased delivery of substrate (FFA) to the liver. In support of this hypothesis are the findings of Schade and Eaton(150) that elevations of blood FFA induced by heparin caused a prompt increase in blood ketone levels in normal subjects. Additional support it derived from the observations of Heimberg et al. (151) that, in the perfused rat liver, ketone production is directly proportional to the concentration of FFA in the perfusate between 0 and 2.5 mM. Other workers, however, have presented evidence to show that increased hepatic delivery of FFA is necessary, but not sufficient, to cause increased hepatic ketone production (152). McGarry and Foster (153-154) postulated that, in addition to elevated blood FFA levels, hepatic ketogenesis must be stimulated, possible through the activation of carnitine acyl

transferase, the enzyme responsible for transport of fatty acids to the mitochondrial oxidative pathway. Furthermore, they suggest that elevations of plasma glucagon levels, or decreases in the insulin/glucagon ratio, regulate the ketogenic activity of the liver. In our study, glucagon levels decreased whereas the insulin/glucagon ratio increased and, therefore, could not be responsible for augmenting hepatic ketogenesis. Our results do not exclude a direct effect of GH on hepatic ketogenesis and further studies will be needed in order to clarify this issue. Of interest in this regard, are studies suggesting that GH may increase ketosis in insulin-deficient diabetics by stimulating the ketogenic capacity of the liver and/or by decreasing ketone removal (89). Regardless of the mechanism involved, the elevated blood FFA and ketone levels induced by GH are consistent with the possibility that GH is a physiologically significant factor in the genesis of stress-induced elevations of FFA and ketone concentrations.

In man, pharmacologic doses of GH cause a decrease in blood amino nitrogen concentrations (129-131) and a decrease in the blood levels of several amino acids (133) within 30 to 60 minutes after administration of GH. In rats, large doses of GH cause increased uptake of many amino acids (119-120) and increased incorporation of most amino acids into protein (120) in muscle and hepatic tissue within 30 to 60 minutes after exposure to GH. In contrast to this rapid, more generalized effect on amino acid metabolism, our data show that physiologic elevations of plasma GH levels result in a specific decrease in plasma alanine con-

centrations that is not apparent until 3 hours after initiation of GH treatment. We found no evidence of a generalized hypoaminoacidemic action of GH which suggests that the results observed after administration of large doses of GH are a pharmacologic, not physiologic, action of GH.

Alanine is of particular importance as the major amino acid precursor utilized for hepatic gluconeogenesis and in normal postabsorptive subjects accounts for a minimum of 6% to 12% of hepatic glucose output (155). The glucose-alanine cycle describes the interorgan transfer of alanine. In brief, alanine is synthesized in muscle from glucose-derived pyruvate by transamination and released into the bloodstream where it is taken up by the liver and converted into glucose (155). The observed reduction in plasma alanine levels could be the result of either a decrease in alanine synthesis and output by muscle or an increase in hepatic alanine uptake. Our data do not distinguish between these possibilities. However, in a study in which pharmacologic concentrations of GH were achieved (200 ng/ml), no effect on hepatic uptake of alanine was observed (107). Thus, it is unlikely that GH-induced changes in hepatic alanine uptake were the cause of the observed decrease in plasma alanine concentration.

Insulin is known to lower the blood concentrations of most amino acids, particularly the branch chained ones, due to an inhibition of their output from muscle (156). In contrast, insulin tends to increase the output of alanine from muscle due to an increase in the synthesis of alanine from glucose (155). Since no

significant changes in the concentration of other amino acids were observed in this study and since insulin tends to increase alanine synthesis in muscle, it is unlikely that the decrease in alanine seen in this study is explained by the elevated plasma insulin levels in the GH-treated subjects.

It is possible that the decrease in plasma alanine concentration was not a direct consequence of GH action on amino acid metabolism. Sherwin et al. (157) have shown that infusion of ketones for 3 hours into normal postabsorptive subjects results in a 21% decline in plasma alanine levels without significantly effecting the concentrations of other amino acids. The steady state concentration of ketones achieved in that study is similar to the concentration observed at the end of the basal period in the present study. Alanine was noted to decrease significantly by 90 minutes after the start of the ketone infusion. In the present study, FFA levels were significantly elevated by 180 minutes, whereas alanine levels did not significantly decrease until 240 minutes after the start of the GH infusion. Thus, our data are consistent with the possibility that the decrease in plasma alanine may be due to an increase in ketones. Additional studies will be needed to determine if the fall in plasma alanine is a direct effect of GH or a secondary consequence of changes in other substrates of hormones, e.g. ketones.

Our studies indicate that physiologic doses of GH produce a rapid, albeit small, increase in basal insulin concentration and a marked increase in glucose-stimulated insulin secretion. Similar findings have previously been reported in situations of chronic GH excess, such as acromegaly (55,57,61-62), and after administration of pharmacologic amounts of GH (62,64-65,106).

Adamson et al, however, have reported an acute decrease in basal and glucose-stimulated insulin secretion after infusion of physiologic quantities of GH (109,113-114). It is difficult to reconcile their findings with our own and with most of the rest of the literature.

The occurrence of increased FFA and ketone levels and the deterioration of glucose tolerance in the face of elevated plasma insulin concentration, suggests that GH may have induced a state of insulin resistance. Chronic GH excess (53-54, 63-65) and acute exposure to pharmacologic doses of GH (64-65,89) have previously been shown to induce insulin resistance. Our results suggest that GH-induced insulin resistance can occur rapidly after physiologic elevations of GH levels.

One possible mechanism of GH-induced insulin resistance is the presence of changes in the insulin receptor, since insulin, like other peptide hormones, must bind to a specific receptor on the cell membrane in order to be effective. The importance of changes in the receptor is emphasized by the many studies showing corresponding changes in the insulin receptor and in insulin sensitivity. For example, insulin binding and insulin sensitivity are both decreased in obesity (158-160), maturity onset diabetes (161-162), uremia (162), and in patients with idiopathic GH deficiency after treatment with GH (163). Conversely, insulin sensitivity and insulin binding are both increased in anorexia nervosa (164-165) and in idiopathic GH deficiency (163). This correlation is not universal, however,

since opposite changes occur in binding and insulin sensitivity in starvation (160), pregnancy (166) and states of high carbohydrate intake (167).

It is not clear exactly what role the insulin receptor plays in GH-induced insulin resistance. In rats, Kahn et al. (102) found that 5 days of treatment with pharmacologic doses of GH caused a slight decrease in number and and increase in affinity of the hepatocyte insulin receptor. Since the net result was normal insulin binding at basal insulin levels, they concluded that GH exerted its anti-insulin actions at a site distal to the insulin receptor. Muggeo et al.(66) similarly found a decreased receptor concentration and an increased receptor affinity in monocytes taken from acromegalic patients. These changes resulted in normal binding of insulin at basal insulin concentrations and decreased insulin binding at high plasma insulin levels. Since many patients with acromegaly have elevated fasting and glucose-stimulated insulin levels (55-61), the insulin receptor might contribute, at least in part, to the insulin resistance of acromegaly. Not all situations of GH excess are associated with decreased insulin sensitivity and decreased insulin binding. Patients with anorexia nervosa, for example, exhibit elevated plasma GH levels, increased insulin sensitivity and increased insulin binding (164-165).

The presence of alterations in insulin binding characteristics and GH excess do not necessarily imply that GH exerts a direct effect on the insulin receptor. Insulin has been shown to

modulate its own receptor both in vitro (168) and in vivo (169). Thus, GH could cause decreases in insulin binding by one of several mechanisms. It could act directly on the insulin receptor, it could block insulin action at a site distal to the receptor, leading, in turn, to hyperinsulinemia and subsequent insulin-induced receptor changes, or it could directly stimulate insulin secretion with insulin secondarily modifying the insulin receptor so as to cause insulin resistance.

In order to see if GH excess acutely effected the insulin receptor, we measured insulin binding to monocytes and erythrocytes at 0 and 4 hours after the start of the GH infusion. This work was done in collaboration with Dr. Vijay Soman. Preliminary results are described in Table 7. They show that specific binding of insulin to erythrocytes decreased by 16% (0 hours, 7.7 ± 1.1% vs 4 hours, 6.5 ± 1.0%, $p<0.01$), whereas insulin binding to monocytes increased by 45% (0 hours, 10.9 ± 0.9% vs 4 hours, 15.5 ± 1.6%). Since only 3 patients were studied, the monocyte data do not reach statistical significance. Preliminary analysis of the binding curves by Dr. Soman suggests that the decrease in insulin binding to erythrocytes is primarily due to a decrease in receptor affinity, whereas the increase in insulin binding to monocytes is mainly due to an increase in receptor affinity, not receptor number.

While very preliminary, these results are the first study in man in which insulin binding was found to decrease in one and increase in another cell type. Previous studies have found that insulin binding to both erythrocytes and monocytes decreases in maturity onset diabetes (161-162) and increases in anorexia

TABLE 7

EFFECT OF GROWTH HORMONE ON INSULIN BINDING

	Monocytes			Erythrocytes		
	0 hr	4 hr		0 hr	4 hr	
Percent bound/			Percent bound/			
$1x10^7$ monocytes/ml	10.1	13.1	$3.5x10^9$ RBC/ml	3.9	7.8	
	11.2	18.5		4.4	4.6	
	11.5	15.1		7.5	5.6	
				5.5	4.6	
				8.6	7.4	
				6.0	4.3	
				13.4	11.4	
Mean	10.9	15.5	Mean	7.7	6.5	$p<0.01$
SEM	±0.4	±1.6	SEM	±1.1	±1.0	

nervosa (164-165). If further studies confirm these results, then the relationship of insulin binding to elements of peripheral blood to insulin binding on insulin-responsive cells such as adipocytes and hepatocytes must by carefully examined. If the monocyte data is indeed representative of the binding changes on insulin-responsive tissue, then it is unlikely that alterations in insulin binding can account for the observed increase in insulin resistance induced by acute exposure to physiologic increments of GH.

CONCLUDING STATEMENT

It has been postulated that GH has two different types of effects on glucose and fat metabolism: 1) an acute insulin-like effect and 2) an anti-insulin like action seen after longer term exposure to GH. Our results suggest that acute elevations of plasma GH concentrations, to levels normally seen during severe stress, do not cause any insulin-like effects and imply that the acute insulin-like actions of GH are a pharmacologic, not physiologic, effect. Furthermore, these data show that the anti-insulin actions of GH can occur within a few hours after exposure to GH. These findings are consistent with the possibility that acute elevations of plasma GH are of physiologic significance in mediating some stress-induced metabolic changes.

REFERENCES

1. Batstone, G.F., K.G.M.M. Alberti, L.Links, P. Smythe, J.E. Lainge, C.M. Ward, D.W. Ely and S.R.Bloom: Metabolic studies in subjects following thermal injury. Intermediary metabolites, hormones, and tissue oxygenation. Burns 2:207-225, 1976.

2. Selye, H., In The Stress of Life, McGraw-Hill, New York. pp. 515, 1976.

3. Eigler, N.,L. Sacca, and R.S. Sherwin: Synergistic interactions of physiologic increments of glucagon, epinephrine and cortisol in the dog. A model for stress-induced hyperglycemia. J. Clin. Invest. 63:114-123. 1979.

4. Martin, J.B.: Neural regulation of growth hormone secretion. N. Engl. J. Med. 288:1384-1392, 1973.

5. Glick, S., J. Roth, R. Yalow, and S. Berson: Regulation of Growth Hormone secretion. Rec. Prog. Horm Res. 21:241-270, 1965.

6. Takahashi, Y., D.M. Kipnis and W.H. Daughaday: Growth Hormone secretion during sleep. J. Clin. Invest. 47:2079-2090, 1968.

7. Unger, R.H.: High growth hormone levels in diabetic ketoacidosis. J. Am. Med. Assoc. 191:945-947, 1965.

8. Frantz, A.G. and M.T. Rabkin: Effects of estrogen and sex difference on secretion of human growth hormone. J. Clin. Endocr. Met. 25:1470-1480, 1965.

9. Merimee, T.J. and S.E. Fineberg: Studies of the sex based variation of human growth hormone secretion. J. Clin. Endocr. Met: 33:896-902, 1971.

10. Merimee, T.J. and D. Rabin: A survey of growth hormone secretion and action. Metabolism 22:1235-1243, 1973.

11. Srivastava, M.C., C.V. Tompkins, J.D.N.Nabarro, and P.H. Sonksen: Studies on the metabolic clearance rate, apparent distribution space and plasma half-disappearance time of unlabelled human growth hormone in normal subjects and in patients with liver disease, renal disease, thyroid disease and diabetes mellitus. Europ. J. Clin. Invest. 3:284-294, 1973.

12. Malacara, J.M., C. Valverde, S. Reichlin and J. Bollinger: Elevation of Plasma Radioimmunoassayable growth hormone in the rat induced by porcine hypothalamic extract. Endocrinol 91:1189-1198, 1972.

13. Roth, J., S.M.Glick, R.S. Yalow and S.A. Berson: Secretion of human growth hormone: physiologic and experimental modification. Metabolism 12:577-579, 1963.

14. Roth,J., S.M. Glick, R.S. Yalow and S.A. Berson: hypoglycemia: A potent stimulus to secretion of growth hormone. Science 140:987-988, 1963.

15. Noel, G.L., H.K. Suh, J.G.Stone and A.G. Frantz: Human prolactin and growth hormone release during surgery and other conditions of stress. J. Clin. Endcr. Met. 35:840-851, 1972.

16. Eddy, R.L., P.F. Gilliland, J.D. Ibarra, J.F. McMurry, Jr. and J.Q. Thompson: Human growth hormone release: comparison of provocative test procedures. Am. J. Med. 56:179-185, 1974.

17. Hunter, W.M., C.C. Fonseka and R. Passmore: The role of growth hormone in the mobilization of fuel for muscular exercise. Quart. J. Exp. Physiol. 50:406-416, 1965.

18. Charters, A.C., W.D. Odell, and J.C. Thompson: Anterior pituitary function during surgical stress and convalesence. Radioimmunoassay of blood TSH, FSH, LH and growth hormone. J. Clin. Endcr. Met. 29:63-69, 1969.

19. Newsome, H. and J.C. Rose: The response of human adrenocorticotroph hormone and growth hormone to surgical stress. J. Clin. Endocr. Met. 33:481-487, 1971.

20. Vigas, M., J. Malatinsky, S. Nemeth, and J. Jurcovicaua: Alpha-adrenergic control of growth hormone release during surgical stress in man. Metabolism 26:399-405, 1977.

21. Goschke, H., E. Bar, J. Girard, A. Leutenegger, W. Niederer, M. Oberholzer and G. Wolff: Glucagon, insulin, cortisol and growth hormone levels following major surgery: Their relationship to glucose and free fatty acid elevations. Horm. Metab. Res. 10: 465-469, 1978.

22. Wright, P.D. and I.D.A. Johnston: The effect of surgical operation on growth hormone levels in plasma. Surgery 77:479-486, 1975.

23. Coponschi, G., M. Hartog, J.M. Earll, and R.J. Havel: Effects of various blood sampling procedures on serum levels of immunoreactive human growth hormone. Metabolism 16:402-409, 1967.

24. Greene, W.A., G. Conron, D.S. Schalch, and B.F. Schreiner: Psychologic correlates of growth hormone and adrenal secretory responses of patients undergoing cardiac catheterization: Psychosom. Med. 32:599-614, 1970.

25. Kohler, P.O., B.W. O'Malley, P.L. Rayford, M.B. Lipsett and W.D. Odell: Effect of pyrogen on blood levels of pituitary trophic hormones. Observations of the usefulness of the growth hormone response in the detection of pituitary disease. J. Clin. Endocr. 27:219-226, 1967.

26. Frohman, L.A., E.S. Horton, and H.E. Lebovitz: Growth hormone releasing action of a pseudomonas endotoxin. Metabolism 16:57-67, 1967.

27. Kimball, H.A., M.B. Lipsett, W.D. Odell, and S.M. Wolff: Comparison of the Effect of the pyrogens, etiocholanolone and bacterial endotoxin on plasma cortisol and growth hormone in man. J Clin Endocr 28:337-342, 1968.

28. Beisel, W.R., K. A.Woeber, P.J. Bartelloni, and S.H. Ingbar: Growth hormone response during sandfly fever. J Clin Endocr 28:1220-1223, 1968.

29. Cryer, P.E. and W.H. Daughaday: Diabetic ketosis-serial plasma growth hormone concentrations during therapy. Diabetes 19:519-523, 1970.

30. Gerich, J.E., M.M. Martin and L. Recant: Clinical and metabolic characteristics of hyperosmolar nonketotic coma. Diabetes 20:228-238, 1971.

31. Livesey, J.H., R.S. Scott, and R.A. Donald: Urinary growth hormone in diabetic ketoacidosis. Horm Metab Res 11:142-146, 1979.

32. Sonksen, P.H., C.V. Tompkins, M.C. Srivastava, and J.D.N. Nabarro: Growth-hormone and cortisol responses to insulin infusion in patients with diabetes mellitus. Lancet 2:155-160, 1972.

33. Alberti,K.G. and T.D. Hockaday: Diabetic coma: serum growth hormone before and during treatment. Diabetologia 9:13-19, 1973.

34. Samaan, N.A., D.B. Stone and R.D. Eckhardt: Serum glucose, insulin and growth hormone in chronic hepatic cirrhosis: Arch. Int. Med. 124:149-152, 1969.

35. Hernandez, A,E. Zorrilla and H. Gershberg: Decreased insulin production, elevated growth hormone levels and glucose intolerance in liver disease. J. Lab. Clin. Med. 73:25-33, 1969.

36. Conn, H.O. and W.H. Daughaday: Cirrhosis and diabetes. V. Serum human growth hormone levels in Laennec's cirrhosis. J. Lab. Clin. Med. 76:678-688, 1970.

37. Samaan, N., W.S. Cumming, J.W. Craig and O.H. Pearson: Serum growth hormone and insulin levels in severe renal disease. Diabetes 15: 546-550, 1966.

38. Horton, E.S., C. Johnson and H.E. Lebovitz: Carbohydrate metabolism in uremia. Ann. Int. Med. 68: 63-74, 1968.

39. Wright, A.D., C. Lowy, T.R. Fraser, I.M. Spitz, A.H. Rubenstein and I. Bersohn: Serum growth hormone and glucose intolerance in renal failure. Lancet 2:798-800, 1968.

40. Ramire, G., W.M. O'Neill, H.A. Bloomer, and W. Jubiz: Abnormalities in the regulation of growth hormone in chronic renal failure. Arch Int Med 138: 267-271, 1978.

41. Miyabo, S., T. Asato and N. Mizushima: Prolactin and growth hormone responses to psychological stress in normal and neurotic subjects. J. Clin. Endocr. Met. 44:947-951, 1977.

42. Greenwood, F.C. and J. Landon: Growth hormone secretion in response to stress in man. Nature: 210:540-541, 1966.

43. Kurokawa, N., H. Suematsu, H. Tamai, M. Esaki, H. Aoki and Y. Ikemi: Effect of emotional stress on human growth hormone secretion. J. Psychosom. Res. 21:231-235, 1977.

44. Brown, W.A. and G. Heninger: Cortisol, growth hormone, free fatty acids and experimentally evoked affective arousal. Am. J. Psychiatry 132:1172-1176, 1975.

45 Martin, J.B.: Neural regulation of growth hormone secretion. Med Clin N. Amer. 62:327-336, 1978.

46. Brown, J.M., J.A. Seggie, J.W. Chambers and P.G. Ettigi: Psychoendocrinology and growth hormone: a review. Psychoneuroendocrinology 3: 131-153, 1978.

47. Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guilleman: Hypothalamic polypeptide that inhibits the secretion

of immunoreactive growth hormone. Science 179:77-79, 1973.

48. Martin, J.B., Plasma growth hormone response to hypothalamic or extra-hypothalamic electrical stimulation. Endocrinology 91:107-115,1972.

49. Martin, J.B., Functions of the hypothalamus and amygdala in regulation of growth hormone secretion. Trans. Am. Neurol. Ass. 98:229-232, 1973.

50. Seggie, J. and G.M. Borwn: Coping with stress: parallelism between the effects of septal lesions on growth hormone and corticosterone levels. Biol. Psychiat. 11:583-597, 1976.

51. Davidoff, L.M., Studies in acromegaly III. The anamnesis and symptomatology in one hundred cases. Endocrino. 10:453-483, 1926.

52. Wright, A.D., D.M. Hill, C. Lowy and T.R. Fraser: Mortality in acromegaly. Quart. J. Med. 153:1-16, 1970.

53. Luft, R., E. Cerasi, and C.A. Hamberger: Studies on the pathogenesis of diabetes in acromegaly. Acta Endocrinol. 56: 593-606, 1967.

54. Maneschi, F.,A. Pilo and R. Navalesi: A Kinetic analysis of plasma insulin disappearance and glucose-induced insulin delivery rate in diabetic and nondiabetic acromegalics. Metabolism 28:1011-1022, 1979.

55. Sonksen, P.H., F.C. Greenwood, J.P. Ellis, C. Lowy, A. Rutherford, and J.D.N. Nabarro: Changes of carbohydrate tolerance in Acromegaly with progress of the disease and in response to treatment. J. Clin. Endocr. 27:1418-1430, 1967.

56. Ikkos, D., R. Luft, C.A. Gemzell and S. Almquist: Effect of human growth hormone on glucose tolerance and some intermediary metabolites in man. Acta Endocrin. 39:547-566, 1962.

57. Cerasi, E.and R. Luft: Insulin response to glucose loading in acromegaly. Lancet 2: 769-771, 1964.

58. Beck, P., D.S. Schalch, M.L. Parker, D.M. Kipnis, and W.H. Daughaday: Corelative studies of growth hormone and insulin plasma concentrations with metabolic abnormalities in acromegaly. J. Lab. Clin. Med. 66:366-379, 1966.

59. Fineberg, S.E., T.J. Merimee, D. Rabinowitz, and P.J. Edgar: Insulin Secretion in acromegaly. J. Clin. Endocr. Met. 30:288-292, 1970.

60. Grodsky, G.M. and P.H. Forsham: An immunochemical assay of total extractable insulin in man. J. Clin Invest 39:1070-1079, 1960.

61. Yalow, R.S. and S.A. Berson: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39:1157-1175, 1960.

62. Daughaday, W.H. and D.M. Kipnis: The growth-promoting and anti-insulin actions of somatostatin. Rec. Prog. Horm. Res. 22:49-93, 1966.

63. Galbraith, H-J.B., J. Ginsberg and A. Paton: Decreased response to intra-arterial insulin in acromegaly. Diabetes 9: 459-465, 1960.

64. Zierler, K.I. and D. Rabinowitz: Roles of insulin and growth hormone based on studies of forearm metabolism in man. Medicine (Balt) 42: 385-401, 1963.

65. Rabinowitz, D.and K.I. Zierler: A metabolic regulating device based on the actions of human growth hormone and insulin, singly and together on the human forearm. Nature 199:913-915, 1963.

66. Muggeo, M., R.S. Bar, J. Roth, C.R. Kahn, and P. Gorden: The insulin resistance of acromegaly: evidence for two alterations in the insulin receptor on circulating monocytes. J. Clin. Endocr. Met. 48:17-25, 1979.

67. Birnbaum,R.S. and H.M. Goodman: Studies on the mechanism of the antilipolytic effects of growth hormone. Endocrinology 99:1336-1345, 1976.

68. Birnbaum, R.S. and H.M. Goodman: Comparison of several insulin-like effects of growth hormone. Horm Metab Res. 11:136-142, 1979.

69. Goodman, H.M., early and late effects of GH on the metabolism of glucose in adipose tissues. Endocrinology 76: 1134-1140, 1965.

70. Swislocki, N.I. and C.M. Szego: Acute reduction of plasma-nonesterified fatty acid by growth hormone in hypophysectomized and Houssay rats Endocrinology 76:665-672, 1965.

71. Swislocki, N.I., Effects of nutritional status and the pituitary on the acute plasma free fatty acid and glucose responses of rats to growth hormone administration. Metabolism 17:174-180, 1968.

72. Chernick, S.S., C.M. Clark, R.J. Gardiner, and R.O. Scow: Role of lipolytic and glucocorticoid hormones in the development of diabetic ketosis. Diabetes 21: 946-54, 1972.

73. Fain, J.N., A. Dodd, and L. Novak: Enzyme regulation in gluconeogenesis and lipogenesis-relationship of protein synthesis and cyclic AMP to lipolytic action of growth hormone and glucocorticoids. Metabolism 20:109-118, 1971.

74. Goodman, H.M., Multiple effects of growth hormone on lipolysis: Endocrinology 83:300-308, 1968.

75. Fain, J.N. Effect of dibutyryl-3'-5'-AMP, theophylline, and norepinephrine on lipolytic action of growth hormone and glucocorticoids in white fat cells. Endocrinology 82:825-830, 1968.

76. Goodman, H.M. Antilipolytic effects of growth hormone. Metabolism 19: 849-855, 1970.

77. Lotspeich, W.D., and V.P. Petersen: Effect of anterior pituitary growth hromone on synthesis of acetoacetate by surviving slices of rat liver. Am. J. Physiol. 176:232-238, 1954.

78. Penhos, J.C., C.H. Wu, A. Lemberg, J. Daunas, B. Brodoff and R. Levine: The effect of growth hormone on the metabolism of lipids and

on urea formation by the perfused rat liver. Metabolism 15: 1109-1119, 1966.

79. Altszuler, N., I. Rathgeb, B. Winkler, R.C. DeBodo, and R. Steele: The effects of growth hormone on carbohydrate and lipid metabolism in the dog. Ann N.Y. Acad. Sci. 148:441-458, 1968.

80. Raben, M.S. and C.H. Hollenberg: Effect of growth hormone on plasma fatty acids. J. Clin. Invest. 38:484-489, 1959.

81. Campbell, J. and K.S. Rastogi: Augmented insulin secretion due to growth hormone: stimulating effects of glucose and food in dogs. Diabetes 15:749-758, 1966.

82. Bishop, J.S., R. Steele, N. Altszuler, I. Rathgeb, C. Bjerknes and R.C. deBodo: Diminished responsiveness to insulin in the growth hormone-treated normal dog. Am. J. Physiol. 212:272-278, 1967.

83. Fineberg, S.E. and T.J. Merimee: Acute metabolic effects of human growth hormone. Diabetes 23: 499-513, 1974.

84. Cheng, J.S. and N. Kalant: Effects of insulin and growth hormone on the flux rates of plasma glucose and plasma free fatty acids in man. J. Clin. Endocr. 31:647-653, 1970.

85. Schlach, D.S. and D.M. Kipnis: Abnormalities in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. J. Clin. Invest. 44:2010-2020, 1965.

86. Hollobaugh,S.L., M. Tzagournis, R.L. Folk, F.A. Kruger, and G.J. Hamur: The diabetogenic action of human growth hormone: glucose fatty acid interrelationships. Metabolism 17:485-491, 1968.

87. Davidson, M.B. and J.M. Bernstein: The effect of nicotinic acid on growth-hormone induced lipolysis and glucose tolerance. J. Lab. Clin. Med. 81:568-576, 1973.

88. Rabinowitz, D., G.A. Klasson, and K.L. Zierler: Effect of human growth hormone on muscle and adipose tissue metabolism in the forearm of man. J. Clin. Invest. 44:51-61, 1965.

89. Schade, D.S., R.P. Eaton and G.T. Peake: The regulation of plasma ketone body concentration by counter-regulatory hormones in man. Diabetes 27: 916-924, 1978.

90. Gerich, J.E, M. Lorenzi, D.M. Bier, E.Tsalikian, V. Schneider, J.H. Karam and P.H. Forsham: Effects of physiologic levels of glucagon and growth hormone on human carbohydrate and lipid metabolism: J. Clin. Invest. 57:875-884, 1976.

91. Mahler, R.J. and O. Szabo: Early insulin synergistic activity of growth hormone. Diabetes 18: 550-555, 1969.

92. Goodman, H.M., Effects of growth hormone on glucose utilization in diaphragm muscle in the absence of increased lipolysis. Endocrinology 81: 1099-1103, 1967.

93. Goodman, H.M. Growth hormone and the metabolism of carbohydrate and lipid in adipose tissue. Ann. N.Y. Acad. Sci. 148:419-440, 1968.

94. Milman, A.E., and J.A. Russell: Some effects of purified pituitary growth hormone on carbohydrate metabolism in the rat. Endocrinology 47:114-128, 1950.

95. Larson, B.A., T.L. Williams, U.J. Lewis, and W.P. Vanderlaan: Insulin secretion from pancreatic Islets: Effect of growth hormone and related proteins. Diabetologia 15:139-132, 1978.

96. Tai, T.Y. and S. Pek: Direct stimulation by growth hormone of glucagon and insulin release from isolated rat pancreas. Endocrinology 99: 669-677, 1976.

97. Bornstein, J., J. McD. Armstrong, M.K. Gould, J.A. Harcourt and M.D. Jones: Mechanism of the diabetogenic action of growth hormone. Biochim. Biophys. Acta 192:265-270, 1969.

98. Mills, J.B, C.R. Reagan, D. Rusman, J.L. Kostyo, P. Zachariah and A.E. Wilhelmi: Metabolic effects of plasmin digests of human growth hormone in the rat and man. J. Clin. Invest. 52:2941-2951, 1973.

99. Davidson, M.B. Effect of growth hormone administration to hypophysectomized rats on muscle lipid metabolism. Metabolism 28: 729-734, 1979.

100. Martin, J.M., H.K. Akerblom, and G. Garay: Insulin secretion in rats with elevated levels of circulating growth hormone due to M+T-W15 Tumor. Diabetes 17:661-667, 1968.

101. Martin, J.M. and J.J. Gagliardino: Effect of growth hormone on the isolated pancreatic islets of rat in vitro. Nature 213:630-631, 1967.

102. Kahn, C.R. ,I.D. Goldfine, D.M. Neville and P. DeMeyts: Alterations in insulin binding induced by changes in vivo in the levels of glucocorticoids and growth hormone. Endocrinology 103:1054-1057 ,1978.

103. Kurtz, M.,R.C. DeBodo, S.P. Kiang, and A. Ancowitz: Hypoglycemia produced by purified anterior pituitary growth hormone and its relationship to the pancreas. Proc. Soc. Exp. Biol. Med. 76:21-24, 1951.

104. Altszuler, N., R. Steele, J.S.Wall, A. Dunn and R.C. DeBodo: Effect of growth hormone on carbohydrate metabolism in normal and hypophysectomized dogs; studies with C^{14} glucose. Am J. Physiol. 196:121-124, 1959.

105. DeBodo, R.C., R. Steele, N. Altszuler, A. Dunn, and J.S. Bishop: On the hormonal regulation of carbohydrate metabolism; studies with C^{14} glucose. Rec. Prog. Horm. Res. 19:445-488, 1963.

106. Frohman, L.A., M.H. MacGilliuray, and T. Aceto: Acute effects of human growth hormone on insulin secretion and glucose utilization in normal and growth hormone deficient subjects. J. Clin. Endocr. 24: 561-567 , 1967.

107. Adamson, U., J. Wahren, and E. Cerasi: Influence of growth hormone on splanchnic glucose production in man. Acta Endocrinologia 86:803-812, 1977.

108. Felig, P., E.B. Marliss and G.F. Cahill, Jr.: Metabolic response to human growth hormone during prolonged starvation. J. Clin. Invest. 50:411-421, 1971.

109. Adamson, U and E. Ceresi: Acute suppressive effect of human growth hormone on basal insulin secretion in man. Acta Endocrinol. 79: 474-481, 1975.

110. Fajans, S.S., J.C. Floyd, R.F. Knopf and J.W. Conn: Effect of amino acids and proteins on insulin secretion in man. Rec. Prog. Horm. Res. 23: 619-662, 1967.

111. Yalow, R.S., S.J. Goldsmith and S.A. Berson: Influence of physiologic fluctuations in plasma growth hormone on glucose tolerance. Diabetes 18:402-408, 1969.

112. Rizza, R.A., P.E. Cryer and J.E. Gerich: Role of glucagon, catecholamines and growth hromone in human glucose counterregulation. J. Clin Invest. 64:62-71, 1979.

113. Adamson, U. and E. Cerasi: Acute effects of exogenous growth hormone in man: Time-and-dose-bound modification of glucose tolerance and glucose-induced insulin release. Acta Endocrinol. 80:247-261, 1975.

114. Adamson, U. and S. Effendic: Insulin-like and diabetogenic effects of growth hormone in healthy subjects, diabetics and low insulin responders. J. Clin. Endocrinol. Metab. 49:456-462, 1979.

115. Evans, H.M. and J.A. Long: The effect of the anterior lobe administered intraperitoneally upon growth, maturity and estrous cycles of the rat. Anat. Record 21: 62-63, 1921.

116. Teal, H.M. and H. Cushing: Studies in the physiological properties of the growth promoting extracts of the anterior hypophysis. Endocrinology 14:157-163, 1930.

117. Griffman, A.C., J.M. Luck, V. Kulakoff and M. Mills: Further observation on the endocrine regulation of blood amino acids. J. Biol. Chem. 209:387-393, 1958.

118. Engel, F.L. and J.L. Kostyo: Metabolic actions of pituitary hormones, in The Hormones V. 69-158, 1964.

119. Kostyo, J.L. and D.F. Nutting: Growth hormone and protein metabolism in: Handbook of Physiology. Section 7. Vol. 4. Part 2 187-210, 1974.

120. Knobil, E., The pituitary growth hormone: an adventure in physiology. Physiologist 9:25-44, 1966.

121. Kostyo, J.L. Separation of the effects of growth hormone on muscle amino acid transport and protein synthesis. Endocrinol 75: 113-119, 1964.

122. Kostyo, J.L. and J.A. Rillema: In vitro effects of growth hormone on the number and activity of ribosomes engaged in protein synthesis in the isolated rat diaphragm. Endocrinol. 88:1054-1060, 1971.

123. Florini, J.R. and C.B. Breuer: Amino acid incorporation into protein by cell free systems from rat skeletal muscle. Biochemistry 5: 1870-1877, 1966.

124. Salmon, W.D. and M.R. DuVall: In vitro stimulation of leucine incorporation into muscle cartilage protein by a serum fraction with sulfation factor activity: Differentation of effects from those of growth hormone and insulin: Endocrinol. 87:1168-1180, 1970.

125. Kostyo, J.L. and F.F. Nutting: Acute In vivo effects of growth hormone on protein synthesis in various tissues of hypophysectomized rats and their relationship to the levels of thymidine factor and insulin in the plasma. Horm. Metab. Res. 5:157-172, 1973.

126. Albertsson-Wikland, K. and O. Isaksson: Time course of the effect of growth hormone in vitro on amino acid and monosaccharide transport and on protein synthesis in diaphragm of young normal rats. Endocrinol. 102:1445-1451, 1978.

127. Albertsson-Wikland, K., S. Eden, and O. Isaksson: Analysis of early responses to growth hormone on amino acid transport and protein synthesis in diaphragms of young normal rats: Endocrinol. 106: 291-297, 1980.

128. Albertsson-Wikland, K., S. Eden, K. Ahren, and O. Isaksson: Analysis of refractoriness to the effects of growth hormone on amino acid transport and protein synthesis in diaphragms of young normal rats. Endocrinol. 106:298-305, 1980.

129. Beck, J.C, L.E. McGarry, I. Dyrenfurth, and E.H. Venning: The metabolic effects of human and monkey growth hormone in man. Ann Intern Med 49:1090-1105, 1958.

130. Berggenstal, D.M. and M.P. Lipsett: Metabolic effects of human growth hormone and growth hormone of other species in man. J. Clin Endocr. 20:1427-1436, 1960.

131. Rimdin, D.L., T.J. Merimee, D. Rabinowitz, L.L. Cavalli-Sforza, and V.A. McKusick: Peripheral subresponsiveness to human growth hormone in the african pygmies. N. Eng. J. Med. 281:1383-1388, 1969.

132. Rudman, D., S.B. Chyatte, J.H. Patterson, G.G. Gerron, I. O'Beirne, J. Barlow, P. Ahmana, A. Jordan, and R.C. Mosteller: Observations on the responsiveness of human subjects to human growth hormone. J. Clin. Invest. 50: 1941-1949, 1971.

133. Jover, E., C. Paradinas, and R. Velasco: Metabolic changes induced by human growth hormone. J. Med. 8:415-424, 1977.

134. Washko, M.E. and E.W. Rice: Determination of glucose by an improved enzymatic procedure. Clin. Chem. 7:542-545, 1961.

135. Rosselin, G., R. Assan, R.S. Yalow, and S.A. Berson : Separation of antibody-bound and unbound peptide hormones labelled with iodine-131

by talcum powder and precipitated silica. Nature (Lond) 212: 355-357, 1966.

136. Faloona, G., and R.H. Unger: Glucagon In: Methods of Hormone Radioimmunoassay. B.M. Jaffe and H.R. Behrman, editors. Academic Press, New York, 1974.

137. Wright, D.R., A.D. Goodman, K.D. Trimble: Studies on "big' growth hormone from human plasma and pituitary. J. Clin Invest 54:1064-1073, 1974.

138. Williamson, D.H., J. Mellanby, and H.A. Krebs: Enzymic determination of D(-)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J 82:90-96, 1962.

139. Novak, M., Colorimetric ultramicro-method for the determination of free fatty acids. J. Lipid Res. 6:431-433, 1965.

140. Spackman, D.A., W.H. Stein, and S. Moore: Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 30:1190-1206, 1958.

141. Soman, V., W. Tamborlane, R. DeFronzo, M. Genel and P. Felig: Insulin Binding and insulin sensitivity in isolated growth hormone deficiency. N. Eng. J. Med. 299:1025-1030, 1978.

142. Scatchard, G: The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51:660-677, 1949.

143. Gambhir, K.K., J.A. Archer and L. Carter: Insulin radioreceptor assay for human erythrocytes. Clin Chem. 23:1590-1595, 1977.

144. Snedecor, G.W. and W.G. Cochran: Statistical Methods. Iowa State University Press, Ames, Iowa, 1967.

145. Carey, L.C., T. Cloutier, and B.D. Lowery: Growth hormone and adrenocortical response to shock and trauma in the human. Ann. Surg. 174:451-458, 1971.

146. Felig, P., and R.S. Sherwin: Carbohydrate homeostasis, liver, and diabetes. Progress in Liver Diseases 5:149-171, 1976.

147. Radziuk, J., T.J. McDonald, D. Rubinstein, and J. Dupre: Initial splanchnic extraction of ingested glucose in normal man. Metabolism 27:657-669, 1978.

148. DeFronzo, R.A., E. Ferrannini, R. Hendler, J. Wahren, and P. Felig: Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. Proc. Natl. Acad. Sci. 75:5173-5177, 1978.

149. Randle, J.P., P.B. Garland, C.W. Hales, and E.A. Newsholme: The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1: 785-789, 1963.

150. Schade, D.S., and R.P. Eaton: Modulation of fatty acid metabolism by glucagon in man IV. Effects of a physiologic hormone infusion in normal man. Diabetes 25: 978-983, 1976.

151. Heimberg, M., I. Weinstein, and Miloslav Kohout: The effects of glucagon, dibutryl cyclic adenosine- 3',5'-monophosphate, and concentration of free fatty acid on hepatic lipid metabolism. J. Biol. Chem. 244:5131-5139, 1969.

152. McGarry, J.D., and D.W. Foster: Regulation of ketogenesis and clinical aspects of the ketotic state. Metabolism 21:471-489, 1972.

153. McGarry, J.D., and D.W. Foster: Ketogenesis and its regulation. Am. J. Med. 61: 9-13, 1976.

154. McGarry, J.D., and D.W. Foster: Hormonal control of ketogenesis. Arch. Intern. Med. 137:495-501, 1977.

155. Felig, P., Amino acid metabolism in man. Ann. Rev. Biochem. 84:933-953, 1975.

156. Pozefsky, T., P. Felig, J. Tobin, J.S. Soeldner, and G. Cahill: Amino acid balance across tissues of the forearm in postabsorptive man: effects of insulin at two dose levels: J. Clin. Invest. 48:2273-2282, 1969.

157. Sherwin, R.S., R.G. Hendler, and P. Felig: Effect of ketone infusions on amino acid and nitrogen metabolism in man. J. Clin. Invest. 55: 1382-1390, 1975.

158. Archer, J.A.,P. Gorden, J.R. Gavin, M.A. Lesniak, and J. Roth: Insulin receptors in human circulating lymphocytes: application to the study of insulin resistance in man. J. Clin. Endocr. Met. 36:627-633, 1973.

159. Olefsky, J., Decreased insulin binding to adipocytes and circulating monocytes from obese patients. J. Clin. Invest. 57: 1165-1172, 1976.

160. DeFronzo, R.A., V. Soman, R.S. Sherwin, R. Hendler and P. Felig: Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding. J. Clin. Invest. 62:204-213, 1978.

161. DeFronzo, R., D. Deibert, R. Hendler, P. Felig, and V. Soman: Insulin sensitivity and insulin binding to monocytes in maturity-onset diabetes. J. Clin. Invest. 63:939-946, 1979.

162. Gambhir, K.K., J.A. Archer, T.J. Robinson, L. Carter, C. Bradley and V.W. Hollis: Erythrocyte insulin receptors: characterization in normal, diabetic, and uremic man.

Clin. Res. 415A, 1976.

163. Soman, V.J., W. Tamborlane, R. DeFronzo, M. Genel, and P. Felig: Insulin binding and insulin sensitivity in isolated growth hormone deficiency. N. Engl. J. Med. 299: 1025-1030, 1978.

164. Wachslicht-Rodbard, H., H.A. Gross, D. Robbard, M. H. Ebert, and J. Roth: Increased insulin binding to erythrocytes in anorexia nervosa. N. Engl. J. Med. 300:882-887, 1979.

165. Soman, V.R. and P. Felig: Insulin binding to monocytes and insulin sensitivity in anorexia nervosa. Am. J. Med. 68:66-72, 1980.

166. Soman, V., Heterogeneity of insulin receptor changes in pregnancy. Diabetes 28:393, 1979.

167. Kolterman, O.G., M. Greenfield, G.M. Reaven, et al: Effect of a high carbohydrate diet on insulin binding to adipocytes and on insulin action in vivo in man. Diabetes 28: 731-739, 1979.

168. Gavin, J.R., J. Roth, D.M. Neville, P. DeMeyts, and D.M. Buell: Insulin-dependent regulation of insulin receptor concentrations: A direct demonstration in cell culture. Proc. Nat. Acad. Sci. (USA) 71: 84-88, 1974.

169. Kobayashi, M., and J.M. Olefsky: Effect of experimental hyperinsulinemia on insulin binding and glucuse transport in isolated rat adipocytes. Am. J. Physiol. E53-62, 1978.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE